APPLICATION OF EPIDEMIOLOGICAL METHODS IN HEALTH MANAGEMENT OF FARMED WARM-WATER FINFISH IN CHINA

BY

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APPLICATION OF EPIDEMIOLOGICAL METHODS IN HEALTH MANAGEMENT OF FARMED WARM-WATER FINFISH IN CHINA

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ABSTRACT

Epidemiological methods have rarely been used to study health problems in finfish in China, and there is a need to develop evidence-based approaches tailored to pond aquaculture in this country. The purpose of the thesis is to illustrate how two epidemiological approaches, descriptive and risk factor studies, can be utilized to understand causes of fish health problems occurring along the farm-to-table food chains of live warm-water finfish production.

A farmer survey was used to describe production performance and management practices of yellow-catfish farming along the Yangtze River and Pearl River Delta, the two main freshwater aquaculture areas in China. Survey data were used to estimate farm-level profitability and biosecurity knowledge, practices, and attitudes of yellow catfish farmers. A returns-costs analysis tool was developed for assessment of the net returns of aquaculture producers. High feed and land rental costs negatively affected net returns in two provinces, and the market price of fish was also an influential factor in net returns. Biosecurity survey data from the same study group were used to document perceptions and behaviors of fish farmers with regard to disease control on their farms. Most farmers' practices were not in compliance with the principles of biosecurity, especially in regard to prevention of pathogen introduction and spread, which might be viewed as "the cause of the cause" of fish health problems. The study also showed there was a need to improve farmers' implementation of aquatic biosecurity practices at the farm level through educational efforts.

Time-series regression (TSR) using overdispersed models and cross-classified random effect modeling methods were applied to fish mortality data from aquatic logistics and feed companies. Although biological and diagnostic data were limited, the 2 different modeling methods had similar objectives: namely, to determine anthropogenic factors related to fish mortalities of grass carp farmed in pond systems and those of fish at delivery to wholesale markets.

The main TSR model suggested that the following effects could explain the increase of fish mortality counts: delayed effect of stocking of new fish and increasing water temperature. Treatment with Chinese traditional medicine or probiotics could be effective in reducing fish mortality. Comparing the results of mortality claims for the other 2 species (largemouth bass and Chinese perch), with cross-classified modeling, the models for longsnout catfish indicated that a significant fixed effect of market and customers might be responsible for the greater number of claims variation in this species. However, some variations in mortality claims may be attributed to delivery factors. Both studies highlighted the utility of analysis of farm/production records, which is an essential health management tool for aquatic animal populations.

Given the lack of aetiological evidence for fish health problems, the research in the thesis successfully took snapshots of production and marketing of farmed warm-water finfish in China, using first-hand survey data and industry production data. Context-specific epidemiological approaches were demonstrated to be adaptable for the purposes of describing the social facets of fish production and investigating risk factors for fish mortality. The thesis initiated discussion about how epidemiology applications in warm-water finfish aquaculture could hinge on the inputs of different stakeholders in the country and incorporate evidence-based practices into fish health management.

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“路漫漫其修远兮，吾将上下而求索” **-------** 屈原

(“The road ahead will be long, and our climb will be steep”)

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1.1.1. History, current statistics and needs

China has the earliest recorded history of aquaculture in the world (Rabanal, 1988). More than 2500 years ago, Chinese aquaculturists started the husbandry of warm-water finfish in inland ponds. Based on trial, error, and observation, early traditional fish farmers in China developed pond ecosystems to achieve the production of food fish by relying on natural resources. The ancient, extensive method of Chinese fish culture was regarded mostly as an art, because it required no processed feed and made the best use of a given local ecology and materials (De Silva and Davy, 2009). With its roots in China, the indigenous knowledge of warm-water finfish aquaculture has spread to vast areas in Asia, including Korea, Japan, and many countries in south-east Asia, which has made it possible for farmers in those areas to initiate and develop their own aquaculture practices (McLarney, 1984). The contribution of the Asia-Pacific region to world aquaculture has justified the global importance of Chinese warm-water finfish aquaculture (Bondad-Reantaso and Subasinghe, 2008; Diana et al., 2013b; Wang et al., 2014).

In China, before the 1950s, capture and rearing of wild fish dominated inland fishery production. However, since the late 1960s, with the boost of world aquaculture and introduction of western scientific methods, such as artificial propagation and intensive stocking, warm-water finfish farming in China has seen tremendous changes in both production scales and culture methods (Edwards, 2007; Li et al., 2011). Significant structural shifts in China’s fishery production in the late 1970s have led to significant expansion of inland warm-water fish aquaculture (Zhang and Roteweit, 2004). With an annual growth rate of 5.5% from 2000 to 2012, Chinese warm-water finfish aquaculture production reached 2.33 million tonnes in 2012, which was more than 60% of the global inland aquaculture finfish production (FAO, 2014). This expansion in Chinese freshwater aquaculture has also brought growing pains and the need to think about how to deal with the challenges arising from environmental pollution, food safety, disease outbreaks, and fish health problems (Cao et al., 2015; Mo et al., 2015; Zou and Huang, 2015). With the goal of developing sustainable aquaculture, the Chinese government is now taking concerted actions to address issues related to the negative impacts of rapid expansion (Wang et al., 2014).

The development and refinement of aquaculture skills in China has been taking place over more than 2000 years, and the role played by small-scale fish farming has initiated the conversation between western scientific and oriental indigenous knowledge of fish culture (McLarney, 1984; Briggs, 2005). There are still gaps between the understanding of cultural norms and practices of Chinese aquaculture and the formulation of timely strategies to ensure the appropriate direction and sustainability of fish farming in China. Questions about measurement of fish health problems at population levels and the relationships between biological processes, with their related social and economic concerns, are absent from academic research on domestic aquaculture in China. Due to the lack of recognition of aquatic epidemiology as a valuable approach to Chinese warm-water aquaculture, a wide range of topics, from fish being farmed in ponds to their consumption at the table, need to be initiated to tackle the challenges and take advantage of opportunities in Chinese warm-water aquaculture.

1.1.2. Freshwater finfish species currently cultured in warm-water ponds

China's freshwater fish fauna is diverse, and includes 20 orders, 50 families, and about 900 species. More than 600 species of warm-water finfish are found in China and the most important family is the Cyprinidae, with about 530 species. Farmed freshwater finfish account for more than 90% of total freshwater fishery production, with about 60 species comprising most of the inland farmed finfish.

Broadly speaking, cultured freshwater finfish in China might be differentiated into primary and secondary species, of which the latter could be referred to as brackish-water fishes, farmed in coastal areas. For inland freshwater aquaculture, farmed freshwater finfishes are generally referred to as primary freshwater fish species. According to the National Aquatic Technical Service, finfish species with the highest production are called conventional finfish species, and include grass carp, silver carp, common carp, bighead carp, crucian carp, tilapia, and Chinese bream.

Freshwater finfish culture systems in China include ponds, lakes, rivers, reservoirs, paddy fields, and net cages (Li, 2002; Zhang, 2008). Between 70% and 90% of warm-water finfish farmed in China are reared in freshwater ponds, in small-scale operations, and often clustered in areas conducive to aquaculture (De Silva and Davy, 2009; Li, 2003). Water-water fish species in the thesis refer to those growing optimally at or above 25 ^{o}C (Stickney, 2009). For many domestic aquaculturists in China, the terms “warm-water pond” and “pond” are synonymous with freshwater finfish aquaculture.

The 8 largest warm-water finfish aquaculture production provinces are distributed along the Yangtze River Basin and the Pearl River Basin, and are located in 5 regions in China (Li, 1981): central China (Hubei and Hunan), eastern China (Fujian, Jiangsu, Jiangxi, and Zhejiang), southern China (Guangdong and Guangxi), northern China, and southwestern China. The total aquaculture production in those areas currently accounts for more than 82% of national production (Wang et al., 2014).

1.1.3. Targeted species and the study areas

We targeted 5 warm-water finfish species for our research: (1) yellow catfish, farmed in Zhejiang (Yangtze Delta) and Guangdong Provinces (Pearl River Delta); and other 4 species farmed in Guangdong Province (2) largemouth bass (*Micropterus salmoides*) farmed in Guangdong Province, (3) Chinese perch (*Siniperca chuatsi*), and (4) longsnout catfish (*Leiocassis longirostris*) transported from Guangdong Provinces, and (5) grass carp (*Ctenpharyogodon idella*) cultured in Guangdong Province.

The 5 species are all pond-cultured species, with temperature tolerances ranging from 0 to 39 ℃. Except for grass carp, all the other species are carnivorous and were initially cultured as farmed fish in China in the late 1970s. Among the 4 carnivorous species, largemouth bass is considered an exotic species, while the 2 catfishes and Chinese perch are indigenous species, and distributed in rivers and lakes along the Yangtze and Pearl Rivers. All 4 carnivorous species are monocultured, and grass carp can be either mono- or polycultured. Except for Chinese perch, which feed only on live fish, the other 4 species rely on formulated commercial feed.

1.1.4. Production components of warm-water aquaculture

The main physical inputs of modern pond aquaculture in China are seed, feed, labour, land fertilisers, drugs and chemicals, and fuel and power (Pemsl and Bose, 2008).

Seed and fingerlings: In freshwater aquaculture, "seed" materials refer to eyed eggs and fingerlings (McLarney, 1984). The first section of each production cycle was acknowledged as one of the most important determinants of the success of production centuries ago, when Chinese people started carp aquaculture. Now, except for freshwater eel culture, the seed requirements for all warm-water finfish species are met by artificial propagation (Cheng et al., 2014). Most broodstock populations of current warm-water finfish are sourced from wild fish populations and domesticated at breeding facilities (Hu, 2005). Thus, semi-artificial propagated seeds are incubated and hatched after natural spawning or hormone treatment by selected breeders.

Chinese fish farmers either procure commercially-available seed or carry out husbandry of fertilized eggs, early fry, advanced fry, and fingerlings on their own farms (Woynarovich and Horvath, 1980). Only about 55% of farmers buy the aquaculture seed from government-certified aquatic seed facilities (Fisheries Bureau of the Ministry of Agriculture, 2011).

Feed: Aquatic feed is the major input in most freshwater fish aquaculture systems, and the large volume of warm-water finfish production contributes to China's leading role in global fishmeal utilization (Seager et al., 2009; Chiu et al., 2013). Two types of commercial supplemental feed, pellet and floating, are preferable for most freshwater finfish, and are used together by some farmers. Only a few warm-water finfish species in intensive aquaculture systems rely on live prey fish, i.e. Chinese perch.

The expansion of the aquatic feed industry has been one of the driving forces of aquaculture development in China (Gale, 2015). According to the anecdotal notes from the extension service of aquaculture during our survey, feed conversion ratios and feed prices are often determinants that influence farmers' choice of feed product and company. There are no definitive rules on price differences, but farmers' payment methods might influence variations in feed prices. Farmers normally pay lower prices if they make prepayments of 5-10% of their estimated annual feed cost before stocking, or cash payments after the shipment of feed to their farms. Non-local, trans-provincial feed companies have larger organizational structures and marketing capacities than the local companies, use brand-effect strategies to gain more clients, and may own subsidiary companies. They tend to improve their sales through large sales forces and technology service teams. Local companies are usually much smaller in scale and total marketing volume. Both types of companies need middlemen to assist with sales and service.

Fertilisers: Pond fertilization has been one of the traditional practices in the culture of carp in China (FAO, 1983). Fertilization can assist the pond ecosystem to balance nitrogen and maximize energy utilization. With the transition of traditional extensive fish farming to intensive aquaculture, fertilization still plays an important role in warm-water finfish rearing (Li et al., 2011). Two kinds of fertilisers are used in warm-water aquaculture in China: organic (manures) and chemical fertilisers.

Land: Land rent is currently described as the limiting factor for growth of pond aquaculture in China (Gao, 2013). During the past 30 years, land rent has increased 5-10 fold for fish farmers in the dominant aquaculture areas. The study area in Guangdong belongs to the Pearl River Delta, and is surrounded by areas implementing the national Special Economic Zone (SEZ) policy since economic reforms began in China in the 1980s (Tantri, 2013). One of the expectations of the SEZ is to keep prices low for agricultural products and reduce costs of production, which has dramatically affected the growth and composition of the agricultural sector (Tantri, 2013). Urbanization and the industrial boom have generated intense competition for real estate within the aquaculture industry itself, as well as between aquaculture and manufacturing companies.

The ratio of agriculture land productivity and industrial land is one of the essential variables that cause the conversion of natural ecosystems into agricultural land (Seto & Kaufmann, 2003). Farmers in southern China reportedly choose small farm sizes to avoid the high cost of land rent and the potential risks of agriculture production (Tan et al., 2013). This trend is consistent with the possible inverse relationship between farm size and the profitability of the small-scale farms (Chen et al., 2011).

Labor: Freshwater fish aquaculture is still very labor-intensive in China, and is not as highly mechanized as the salmon industry in other countries (Jerome and Lionel, 2002). It is estimated that about 11 million people work on inland aquaculture in China (Li, 2015). The sector is dominated by small-scale farms, often managed on a household basis, and supported by their relatives and friends and, sometimes, through the use of hired labor, especially for harvesting (Garnett and Wilkes, 2014). Many routine management practices involve heavy work and are conducted by male farmers; some of these activities include feeding fish, pond disinfection, seining, and harvesting.

Aquaculture farmers in China are reported to be overall older than other agriculture workers: more than 80% are between 41-60 years old (Liang and Zhao, 2011). Younger farmers' investments have tended to change from aquaculture to other agricultural practices because of aquaculture's relatively low incomes, labor-intensive nature, and the high risks associated with unstable market prices (Zhang, 2014). For example, in the Pearl River Delta, with the recent competition from industry, aquaculture has gradually become less financially attractive to farmers than it once was. However, this might not be the same case in some parts of the Yangtze River Delta, such as Zhejiang Province, located in the Taihu Lake area, which is one of the earliest freshwater aquaculture areas documented in China's history. In Zhejiang, freshwater fish farming has been an important income source for local farmers for many generations, and the collective skillset and tacit knowledge increase the probability of farmers pursuing aquaculture.

Chemicals are important to control disease, as they reduce losses and increase fish production with the purposes of preventing and curing disease-affected cultured commodities (Jiang, 1996). The chemicals used in aquaculture in China can be divided into 3 types, based on their targeting of different components of the disease triangle: fish host (vaccines, immune-stimulants, probiotics, vitamins, minerals, anti-stressors, Chinese herbs); disease agents (chemotherapeutics, such as antibiotics and antiparasitics); and pond water environment (pond disinfectants, herbicides, and other chemicals for water quality improvement) (Li, 2014).

Common methods for control of infectious diseases and routes for the administration of drugs and chemicals in warm-water aquaculture in China include pond disinfection, water quality improvement, medicated feed, and disinfection of fingerlings or eggs (Jiang, 1996; Noga, 2010).

Pond aquaculture is a closed, intensive system with limited water exchange during the whole production cycle. Once a disease agent is introduced into the pond, it is almost impossible to totally remove it from the system, which makes the culture of animals and plants in an aquatic environment differ significantly from the culture of terrestrial animals. The unique features of pond aquaculture affect the choices of various therapeutic options, since the administration of those drugs and chemicals can simultaneously influence non-targeted organisms in the pond ecosystem, such as other vertebrates, algae, invertebrates, and bacteria.

1.1.5. General management practices at farm-level

Controlled stocking of warm-water fish uses strategies related to stocking density and species combinations, which are important to increase pond productivity and improve utilization of feed resources (Li, 2015). Warm-water aquaculture in China applies traditional principles of polyculture, where different species of varying sizes are reared in the same pond, from fingerling to market-sized fish (Tapiador et al., 1976). Small scale producers make their stocking plans according to their aquaculture experience or guidance from hatchery suppliers (Sharma et al., 1999; Yuan, 2007; Kpundeh, 2013).

Water quality control: Maintaining appropriate water conditions is the most crucial factor affecting the success or failure of intensive pond aquaculture (Lannan et al., 1983), because intensive stocking reduces water quality and increases stress from weather changes, feeding, overcrowding, and other stressors, which may contribute to disease outbreaks (Park et al., 2012). Water quality in pond aquaculture is determined by the physical, biological, and chemical characteristics of pond water, and the combined and continuous variation in these 3 components is termed pond dynamics (Bhatnagar and Devi, 2013). Four water parameters are critical indicators of pond fish health: dissolved oxygen (DO), temperature, pH, and ammonia (Yang, 2007; Yu et al., 2008). Some other water quality factors that likely complicate warm-water culture environments include metabolic waste products other than ammonia (carbon dioxide and nitrite) and the presence of toxicants (heavy metals and organic pollutants) (Knapp, 2010). Compared with cold-water freshwater species, some of the more commonly-cultured warm-water fish species, i.e. common carp and grass carp, are relatively tolerant of poor water quality (Lannan et al., 1983). Two important things need to be considered by farmers when making plans for water treatment: the interaction of different water quality parameters and the cost-effectiveness balance. Since most warm-water fishes are not expensive, the central principle of pond aquaculture investment is to utilize the cheapest physical inputs and rely on nature resources to grow the fish (Boyd and Tucker, 2012).

In China, with the recent introduction of the concept of sustainable and healthy aquaculture, the focus of warm-water aquaculture has shifted from quantity to quality (Zou and Huang, 2015). Water quality management targets cost-effective and eco-friendly pathways to minimize the negative effects of water quality deterioration, such as disinfection of ponds, utilization of aquatic plants, stocking of filter fish, and application of water quality modifiers (Xie, 2010; Ding and Peng, 2014;). Liming and fertilization are traditional methods of improving water quality, with low costs and minimal negative impacts to the pond ecosystems; however, today, they are rarely practiced by young pond aquaculturists in China.

Scientific feeding involves providing feed of the appropriate quality and quantity to the pond at different stages of fish development (Hishamunda and Subasinghe, 2003). Appropriate quality means the proper form for the cultured animals to accept and achieve optimal growth.

Various species in polyculture systems might have different feeding behaviors: 1) thriving on artificial food (e.g., grass carp, black carp); 2) thriving on a diet of both artificial and natural food (e.g., common carp, mud carp); and 3) taking only natural food and no artificial food (e.g., silver carp) (Lannan et al., 1983). In fact, not only polyculture but also monoculture will benefit from a scientific approach to feeding, with an appropriate frequency of feed administration that achieves nutritional effectiveness and avoids overfeeding (Sherpherd, 1995), and which serves the goal of pond management to balance production (anabolism) and decomposition (catabolism) (Bosma and Verdegem, 2011).

Effective pond management planning considers feeding regimen, stocking, fertilization, aeration, and water exchange as combined factors in order to determine the different levels of intensity sustainable by pond systems (Boyd and Tucker, 2012), while accounting for variations of intensity levels among different geographical areas (Edwards, 2008). Given stocking of either monoculture or modern polyculture, farmers with better economic conditions or industrialized farms tend to apply all possible advanced modern technologies in pond aquaculture, such as fertilization, continuous and emergency aeration, and use of commercial supplemental feeds (Edwards, 2015).

Selection of the 3 types of commercial supplemental feeds (powder, pellet, and floating) could be single or mixed (Knapp, 2010). Fish feeders, which are normally installed by aquatic feed providers, are necessary for powder and pellet feeds, but not for floating feed. Feed conversion ratio is an important indicator of the efficiency of feed utilization (Boyd et al., 2007; Chiu et al., 2013); however, it is almost impossible to compare the efficiency of feeding if the evaluation process neglects variations in individual pond management (Boyd and Tucker, 2012; Hong and Xi , 2013).

Disease prevention and control practices in pond aquaculture at the farm level are highly diverse across China. Pond aquaculture farmers in different areas of the country have different levels of knowledge and understanding of disease prevention and control strategies, which is likely affected by their background, proximity to aquatic extension services, and available technology.

The two main principles for warm-water finfish disease management are prevention and treatment. In addition to chemotherapy as an important disease control strategy, ecological methods and vaccination are being encouraged as consistent with the concepts of sustainable aquaculture (Nongbo, 2013). Given the limited resources of government-certified vaccines, preventive measures for fish farmers sometimes refer to other management practices, such as site selection, stocking, water quality control, and feeding (Jiang, 1996). Furthermore, most farmers may not be able to stock with specific-pathogen-free fingerlings or fry to avoid the introduction of pathogens to their farms.

The concept of no antibiotics and environmentally-friendly agents has become increasingly understood and accepted by fish farmers, and the application of probiotics and Chinese traditional medicine has further developed during the past 30 years (Chinabut and Puttinaowarat, 2005; Qi et al., 2009).

Disease diagnosis for warm-water fish aquaculture is achieved by field observations on most small-scale farms in China and other Asian countries (Bondad-Reantaso et al., 2005). Laboratory testing by aquaculture company staff is used for identification and confirmation of disease outbreaks at their own facilities or on their contracted farms.

1.1.6. Live fish from farm to table: transportation and supply chain

Freshwater aquaculture provides 97% of China's domestic food fish. Freshwater fish consumption increased from 4.40 kg per capita per year in 1990 to 13.2 in 2009, in China (FAO, 2011), and the overall market price of freshwater aquaculture products has increased from 8,000 RMB/ton in 2003 to 15,000 RMB/ton in 2012 (Wang et al., 2014).

The domestic markets have lower standards of food safety regulation and levels of enforcement, being separate from the export markets governed mostly by higher international safety and quality standards (Broughton and Walker, 2010). With increasing living standards and a growing middle class in the developing world, including China, that has an appetite for new food varieties, one could expect a change in the species profile of cultured species. This might include a significant increase in carnivorous species, which often are purported to have a better taste and higher demand. In spite of such changes, the backbone of Chinese freshwater aquaculture is likely to continue to be carp polyculture.

Except for tilapia, warm-water species are targeted for domestic consumption, and there is a strong preference for live purchase of freshwater fish for domestic consumption. This is the norm in both rural and urban areas (Hanson et al., 2011; Chiu et al., 2013). Sixty percent of aquatic products in China are distributed by individual vendors, both in markets and on the street, close to the farms where the fish are produced (Zhou et al., 2008), while in wealthier coastal regions, especially southern China, several high-value warm-water fish species are shipped long-distance to many cities in China through a live fish transport supply chain.

For warm-water aquaculture in China, fish are collected by middlemen or fish brokers after harvest. Fish are transported in water tanks installed in trucks, with oxygen provided and sometimes also with ice added to the water. The harvested fish are then shipped either directly to the nearby wholesale market, or to fish processing plants for transportation. There are another 4 steps of live fish being handled between initial transport and final destination at the wholesalers: (1) fish sorting, grading, and holding; (2) live fish being packaged; (3) live fish transportation; and (4) fish transaction. Live fish transportation is one important technological hurdle that limits the expansion of aquaculture (Knapp, 2010).

1.2. Health problems and diseases of warm-water finfish in China

The health of fish is one determinant of the success of intensive aquaculture. Various biotic and abiotic parameters in the culture system can break down the balance of the population immunity (Lipton, 1994). Intensive farming of warm-water finfish in China has been given due attention with regard to aquatic diseases and health problems that result in large scale mortalities, concomitantly reducing production significantly (Li, 2003; Bondad-Reantaso et al., 2005; Wang et al., 2014).

1.2.1. General introduction to diseases of famed warm-water finfish

Four aspects of Chinese warm-water aquaculture have been identified, with regard to the characteristics of aquatic infectious diseases (Chen, 2007; Zeng, 2010):

(1) Common diseases of conventional cultured species are endemic, with high prevalence (i.e., 30% of farms affected in northern China) and mortality (more than 50%), from such diseases as grass carp hemorrhagic disease, bacterial diseases of grass carp, and hemorrhagic septicemia;

(2) Outbreaks of emerging aquatic animal diseases are continuously reported in newly cultivated, high-value species, i.e. tilapia, yellow catfish, and Chinese perch;

(3) Water pollution and the degradation of water quality are regarded as important precursors of disease outbreaks;

(4) Other external factors and management practices might also have significant impacts on water quality, such as natural disasters or improper use of chemotherapeutic products.

In pond aquaculture, there are multiple factors responsible for the occurrence of emerging fish diseases, such as seed quality, environment conditions, and management practices. Oversimplification of the component causes of fish diseases is misleading because it ignores how host, agent, and environmental factors interact (Okamura and Feist, 2011). Management practices in intensive aquaculture operations are potential drivers of evolution of the virulence of aquatic infectious agents, and include (i) rearing at high densities, (ii) compression of rearing cycles, (iii) use of broodstock with limited genetic diversity, and (iv) pathogens causing endemic diseases adapting to the cultured populations (Kennedy et al., 2015). Environmental conditions also play important roles in disease emergence in freshwater ecosystems, due to the introduction of new diseases when using wild fish as broodstock or the environmental degradation that occurs when freshwater resources are over-exploited (Engering et al., 2013).

1.2.2. Aquatic disease agents of farmed warm-water finfish in China

As in other intensive fish farming settings, diseases of warm-water finfish in China can be classified as:

(1) Infectious diseases caused by microorganisms, such as protozoa and metazoa, bacteria, fungi, or viruses.

(2) Non-infectious diseases which are not caused by microorganisms. Factors responsible for non-infectious diseases include deficiencies, intoxications, low oxygen levels, and gas bubble diseases (Brown and Gratzek, 1980). Sudden epidemics are often the result of combined actions of the biotic factors and adverse environmental factor(s) prevailing in the culture system, and are known as “stress-mediated diseases” (Lipton, 1994).

1.2.2.1 Aquatic infectious diseases

Bacterial diseases are the most serious impediment to pond aquaculture production (Huang, 1996). The most prevalent bacterial diseases and their possible primary pathogens include septicaemia (*Aeromonas hydrophilia,* or *Yersinia ruckeri),* furunculosis (*Aeromonas salmonicida),* bacterial gill disease (*Columinaris or Aeromonas sobria*), enteric septicaemia (*Aeromonas punctata*), edwardsiellasis (*Edwardsiella tarda*), ulcer disease or carp erythrodematitis, red skin disease (*Pseudomonas fluorescens),* skin rot disease (also called printing disease, caused by *Aeromonas puncatata),* white skin disease (*Myxococcus piscicola*), and epizootic ulcerative syndrome (Teng et al., 2015; Zhao et al., 2012).

Viral diseasess are associated with many health problems of numerous warm-water finfish species in China. Several aquatic viruses have received attention from both the aquaculture production and academic sides (Wu et al., 2009; Crane and Hyatt, 2011; Sahoo and Goodwin, 2012): Herpesviral Hematopoeitic Necrosis of Goldfish Virus (HVHNV), Grass Carp Reovirus (GCRV), Spring Viremia of Carp Virus (SVCV), and host-specific Cyprinid herpesviruses -1-3 (CyHV-1, CyHV-2, and CyHv-3).

Parasitic diseases: More than 50 kinds of parasitic diseases are reported in China's cultured fishes, some of which are endemic in freshwater finfish species (Cheng, 2008; Ogawa, 2011). Protozoan diseases include crytobiosis, myxosporidiosis, chilodonelliasis, and ichthypoththiriasis; and metazoan diseases include dactylogyriasis, sinergasilliasis, ichthyoxeniosis, diplostomumiasis, and bothriocephalus gowkongensis.

Fungal diseases are not reported as frequently as many of the above types of diseases. Saprolegniasis and branchiomycosis are the most common fungal diseases (Wu et al., 2009; Zhang, 2014).

1.2.2.2 Non-infectious diseases of warm-water finfish aquaculture

Water-quality-related health problems are common non-infectious diseases of warm-water aquacultured fish, and can be caused by (1) water quality change after stocking, possibly indicated as low dissolved oxygen; (2) water source deterioration and degradation of the aquatic environment because of the introduction and accumulation of toxic algae; and (3) chemical pollutants, such as heavy metals, pesticides, and other toxicants (Huang, 1996). Other non-infectious diseases could refer to genetic degradation and nutritional disorders, the latter of which might include under-nutrition and malnutrition, and can predispose fish to infection.

1.2.3. Negative impacts of aquatic diseases in warm-water aquaculture in China

All populations of organisms, including humans, are limited partially or completely by diseases in their ecosystems (Real, 1996). Similar principles apply to modern warm-water aquaculture worldwide, and the complexity of diseases affected by environmental factors and their interactions in populations and ecosystems make tracking and causative assessment of individual diseases extremely difficult.

The economic loss to aquatic diseases in the country, in 2011, was estimated at approximately USD 593.3 million (Bureau of Fisheries of Ministry of Agriculture of China, 2013). Infectious diseases cause the greatest economic loss in high-value species, e.g. Chinese perch. At the farm level, fish disease is a substantial source of monetary loss for aquaculturists, because production costs are increased by fish disease outbreaks and the investment is lost through dead fish, cost of treatments, and decreased growth.

Most related species are facultatively pathogenic and might be isolated from fish without apparent clinical signs or mortality. Fish disease agents sometimes can be transmitted through the aquatic environment and become infective or cause food toxicity, and thus have very important public health implications (Novotny et al., 2004).

1.2.4. Disease information collection of fish farms and aquatic industries

1.2.4.1. Fish disease surveillance and reporting system

Legislative and institutional framework: The Chinese government has established laws and regulations to ensure food quality and safety in China, which involves resources, environment, ecological protection, and food safety (Li et al., 2011). The aquaculture industries are largely regulated by the Fisheries Law and other specific laws, rules, regulations and administrative acts, which have attached importance to the “green” growth of aquaculture (Zou and Huang, 2015).

The national fishery administration has overlapping functions among different ministries, and its general supervision is empowered by the State Council, among which the Chinese Ministry of Agriculture (MoA) is the highest administrative body (Broughton and Walker, 2010; Zhang, 2008).

For aquaculture products for domestic consumption, the Bureau of Fisheries of the Ministry of Agriculture is responsible for national disease prevention and control of all registered aquaculture farms supplying domestic markets (European Commission, 2014). A national system of fish disease monitoring has been in place since 1999, but it only covers about 5% of the total farming area. There are more than 4000 “monitoring points,” which are contact points for farmers to get advice in connection with endemic diseases or to request visits by officials of the Animal Disease Prevention Control Center. In 2004, the MoA started an active surveillance system for aquaculture in the National Aquaculture Animal and Plant Health Monitoring Program, which only covers koi herpes virus disease in warm-water aquaculture. Regional and local fishery bureaus are in charge of implementation of national strategies and plans targeted for industrialized aquaculture farms, but not for small-scale farms.

<u>Technology support systems</u>: The following national-level government agencies, which are subordinate to the MoA, lead the provision of technology extension and support services to aquaculture sectors: (1) National Fishery Technology Extension Centre, which is responsible for new and advanced fishery technology extension and popularization, and is affiliated with the National Introduction and Breeding Center of Aquatic Seeds and the National Aquaculture

Disease Control Centre; (2) China Society of Fisheries, which mainly targets statistics and academic exchange; and (3) China Academy of Fisheries Science, with its institutions spread around the whole country and involvement in almost all research fields relevant to the aquaculture sector.

1.2.4.2. Production & mark records and farm-level logbook records

Aquaculture records are a reliable source of information to trace the health history of fish on a farm, quantify production losses over time, determine their cause(s), and identify management factors to proactively mitigate losses (Pomeroy, 2003; Dewey, 2008; Labrie et al., 2008; Thrush et al., 2012; Serfling, 2015). In many Asian countries, aquaculture record-keeping is still regarded as inadequate, as practiced by small-scale fish farmers, and not standardized on relevant parameters such as daily mortality, feed consumption, and water quality (Bondad-Reantaso et al., 2005;Tan et al., 2006; Marschke and Wilkings, 2014).

Farm records are usually kept in paper format, comprised of daily data on fish stocking, feed intake, water quality, biomass, disease treatments, harvesting of fish, labor inputs, and so on. Record-keeping is also an important health management practice for fish logistics companies, with the focus on transaction records to determine profitability of live fish sales.

Farm records are more strongly encouraged to be kept on 3 kinds of warm-water fish farms in China:

(1) Demonstration farms, certified by the MoA, with the themes of “ecological, healthful, circulatory, and intensive” (Zou and Huang, 2015). To achieve the qualification of demonstration farms, farmers need to ensure that standard production procedures are adopted, with various data being efficiently recorded.

(2) Cooperative farms, belonging to warm-water aquaculture companies for producing marketable fish. Farmers renting cooperative farms need to follow the seedling plan, management measures, and vaccination schedules suggested by the company. Those aquaculture companies require farmers to keep standardized farm records in order to increase the traceability of their product (Ortega et al., 2015).

(3) Farmers are encouraged by their aquatic feed providers to keep farm records using the format recommended by those feed companies. Farmers will get discounts on feed prices if they provide complete records for production cycles. The details are very similar to those taken on cooperative farms, and farm records are often checked during extension service staff's routine visits. Final analyses of each production cycle are done by the feed company to assess farm- or pond-level profitability.

Computer database systems are applied to many certified breeding centers. The central government has also initiated a national program of forecasting for aquatic animal diseases. This program, with its adoption across the country in 2016, applies internet, database management, and GIS technology, with the aim of taking early control measures and minimizing aquatic disease losses (Zhang et al., 2009; National Fishery Technology Extension Center, 2015;).

1.2.4.3. Quality control and validation of fish health data

According to the latest audit carried out by the European Commission, the Chinese MoA exchanges monitoring information with the Administration of Quality Supervision, Inspection, and Quarantine (AQSIQ), which is responsible for all aquaculture products intended for export. The disease monitoring list was defined by the MoA using "A-B-C" disease system defined by World Animal Health Organisation (OIE), which is no longer in use. The MoA is not in charge of transmission of non-compliant sampling test results to the local Entry/Exit Inspection and Quarantine Bureaus (CIQs). However, local agricultural departments are expected to share food safety-related monitoring information with local CIQs through a local government-established information exchange platform, with results to be published online (European Commission, 2013). For viral diseases of warm-water fish in China, local aquaculture agencies are not able to carry out confirmatory diagnoses, and samples need to be sent to Shenzhen CIQ, where the OIE reference laboratory is located.

Rural fish veterinarians are the major workforce dealing with fish disease diagnosis and treatment in China. The national licensed training program for fish veterinarians, starting in Guangdong in 2007, was seen as a neoliberal program for cultivating rational and responsible veterinarians, which redistributed rather than minimized risks that result from market-oriented aquaculture production (Huang, 2015). One positive result from this program is the prescription system which requires fish veterinarians to get licenses if they want to issue prescriptions. Standardized prescription records are required to be kept on file for 2 years, including disease diagnosis records and drug prescriptions.

Diagnoses of fish diseases on rural farms are usually done by fish veterinarians, and the final diagnoses are based on common clinical signs observed under field conditions. In the future, licensed fish veterinarians are expected to use laboratory equipment to detect and identify pathogens.

1.3 Gap and issues

1.3.1. Challenges for farmers and warm-water farmed finfish companies

The challenges for Chinese warm-water aquaculture are mostly due to negative impacts of environment conditions (Song et al., 2012; Misganaw et al., 2015), and various technology bottlenecks to management of those fish health-related risks (Chen, 2014).

1.3.1.1. Climate change

The impact of climate change on aquaculture in China can be related to temperature, precipitation, drought, storms, and floods, among which the variability in mean temperature could have the biggest negative impact (Li et al., 2014). The annual mean temperature in China could increase by 1.5-2.2 ^{0}C by the year 2020, and by 2.3 - 3.3 ^{0}C by 2050 (Ding et al., 2007). Although there is a paucity of studies on the impact of climate change on aquaculture production in China, most expected effects of climate change on aquaculture might be either directly or indirectly related to fluctuations of temperature, followed by heat stress on fish (Holst, 2013).

1.3.1.2. Water quality degradation

With the development of urbanization and the shrinkage of agriculture production, the competition for land from other types of agriculture production and environmental pollution have been compromising the quality and security of water sources (Cao et al., 2007). The eutrophication in water bodies adjacent to land-based pond aquaculture sites is another important concern, and might be related to the discharges from aquaculture ponds, livestock farms, or industries (Cai et al., 2013; Cheng et al., 2013). Furthermore, utilization of aquatic feed and organic fertilizers, both rich in nitrogen, can cause pond pollution through an imbalance of nitrogen, followed by the accumulation of toxic levels of ammonia and nitrite (Song et al., 2012).

High-density reared fish populations challenged by those environmental disturbances are likely to be immunosuppressed (Baldwin, 2010; Tort, 2011; Kpundeh et al., 2014) and, hence, susceptible to acute and chronic stressors, such as infectious agents, therapeutic treatments of diseases, or handling of fish (Tort and Mackenzie, 2002; Davis, 2006). Because the non-specific immune responses of piscine animals to stressors are mostly the opposite of those found in higher vertebrates, the consequence of stressors are generally regarded as detrimental to the fish (Håstein et al., 2005; Anttila et al., 2013; Aerts et al., 2015).

1.3.1.3. Technology bottlenecks

Disease prevention and control have been limiting the stability and expansion of the Chinese warm-water aquaculture production (Chen, 2014). With the goal of increasing survival rates and growth in intensive systems, aquatic technology has been under development in China since the 1980s, such as fish breeding programs, aquatic engineering and nutrition enhancement, and innovative non-antibiotic-based therapeutics (Leung et al., 2007; Gjedrem et al., 2012; Mo et al., 2015; Zou and Huang, 2015). However, given the challenges of introducing new cultured species, and emerging aquatic diseases, current scientific technologies are still unable to meet the needs of aquatic disease control in warm-water finfish (Bondad-Reantaso et al., 2005; Stentiford et al., 2012). According to anecdotal reports from the fish farmers we investigated, inbreeding of broodstock might decrease disease resistance and stress tolerance, which has also been verified as a concern for shrimp production in Asia.

1.3.1.4. Increase of production costs and volatilities of fish price

Aquaculture may be the sole income source for most warm-water finfish farmers in China. As intensive aquaculture is usually a high-risk enterprise, a failed harvest might leave a farmer with no alternative income (Lehane, 2013). For those fish farmers not suffering from economic loss due to fish diseases or health problems, another concern is their farm profit and the production costs of feed, land, and labor. For example, the scarcity of land now, in the rural areas in China, has caused rising rental costs for cultivated land (Gao et al., 2012).

The market price of most warm-water finfishes is not stable in China, which might be influenced by the supply of fish and other animal-origin food products targeting domestic consumption.

1.3.2. Lack of knowledge on several key concepts of fish health management

1.3.2.1. Aquatic biosecurity

Biosecurity, a term originally from agriculture production, has been defined as the process and objective of managing biological risks associated with food and agriculture in a holistic manner. It was also defined for disease management of livestock animals as the probability of avoiding introduction or re-introduction of disease agents to a farm (Stott et al., 2003). Due to the diversities of aquaculture systems, aquatic biosecurity can mean different things to different stakeholders. Generally speaking, to protect the aquaculture investment from preventable losses, the goal of excluding infectious agents and reducing stress can be realized through “an essential group of tools for the prevention, control and eradication of infectious disease and the preservation of human, animal and environmental health” (Lee and O' Bryen, 2003).

Why is aquatic biosecurity important to aquaculture? Biosecurity can be in the form of farm management practices that set up external barriers or internal barriers to biological hazards (Smith, 1998) that help increase profits and improve the prudent and responsible use of veterinary medicine in aquatic food production. At regional and national levels, biosecurity can improve the regulatory environment and assist the legislation and policy development process to enhance the regional and national reputation of food safety; and, no less important, it is a mandatory measure, defined by OIE, for any region or country which declares freedom from specific aquatic pathogens.

With its development for more than 10 years in aquaculture, the modern approach to aquatic biosecurity is risk analysis, which offers an effective management tool where pragmatic decisions can be made to balance competing environmental and socio-economic (Hine et al., 2012). However, this tool requires research on fish disease, fish disease surveillance databases, and other vital sources of information and knowledge.

Biosecurity in the real world can be more than just measures and methods, and biosecurity levels in aquaculture facilities might be more related to the mindsets of the farmers, field operators, and veterinary company managers (Toma et al., 2013; Wauters et al., 2014). It is a mindset prepared against potential, manageable disease risks occurring in aquaculture settings, especially closed, intensive systems. A sound biosecurity program can only be possible if it has been based on a thorough understanding of diseases and their epidemiology (Peeler, 2005).

However, aquaculturists and even academic researchers involved in warm-water aquaculture in China are not aware of the concept and its benefit to intensive aquaculture systems. Only recently have a few aquatic scientists working in disease prevention of shrimp introduced the biosecurity concept to shrimp farmers. There are some reasons for the lack of awareness of biosecurity in pond aquaculture in China, even though it was introduced to salmonid aquaculture in the 1970s:

(1) The conventional aquaculture methods of multiple stocking and harvesting, and the characteristics of open or semi land-based pond systems;

(2) Because the terms biosecurity and biosafety translate from English to Chinese with the same character, many aquatic professionals are not aware of the concept of biosecurity;

(3) Very few publications have reported quantitative analyses on the social and economic impacts of biosecurity on pond aquaculture;

(4) Lack of routine monitoring and surveillance data to support the identification of scientifically sound and cost-effective biosecurity measures.

1.3.2.2. Fish stress and its indication in health management of warm-water finfish

All vertebrates, including fish, rely on stress responses to perform necessary life functions (Schreck, 1981). Stress can be understood as "physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of insult" (Schreck, 2000). The effect of stress in fish can be immediate or accumulated over the long term (Davis, 2006; Marcel et al., 2009; Rapp et al., 2012). Stress can directly or indirectly cause osmoregulatory dysfunction in fish (Carmichael et al., 1984; Weirich et al., 1992; Barton, 2002), which can be sub-acutely or acutely lethal (Tomasso et al., 1980).

Stressors related to fish health can occur during the whole processes of fish farming, starting with harvesting on farms, handling, packaging, loading, transporting, and unloading at destination markets (Barton and Iwama, 1991; Conte, 2004; Sung et al., 2011; Stendera et al., 2012). During the periods before and after transport of live fish, there might be a series of events which can include handling, crowding, and thermal stress (Portz et al., 2006; Hjeltnes et al., 2008). For closed transport with high fish density and low specific water flow, critical water quality parameters (e.g. temperature, dissolved oxygen, carbon dioxide , total ammonia nitrogen, and foam formed by dissolved organic compounds) could become the most common physiological stressors (Hjeltnes et al., 2008).

The global expansion of intensive aquaculture production has raised the importance of stress management, with the focus on improving immune responses of fish towards physical, chemical, and biological stressors (Van Weerd and Komen, 1998; Ruane et al., 2002; Dobsikica and Svobodova, 2009; Tort, 2011; Aerts et al., 2015), which are important in the prevention of losses caused by infections of opportunistic pathogens in the environment (Wendelaar Bonga, 1997; Xia et al., 2014).

However, in the aquatic therapeutics market in China, many commercial “anti-stressor” products claim to efficiently reduce fish stress, despite the lack of sufficient trial data, and are promoted with door-to-door visits at fish farms. Fish farmers might not be able to explore the mechanisms of fish stress through only their own experiences, and the over-usage of commercial products might be more marketing- than science-based, both of which could cause fish farmers to miss opportunities to acquire knowledge useful for fish health management.

1.3.3. Lack of evidence-based research in fish health management in China

The development of warm-water aquaculture in China has raised many concerns about food safety, environmental pollution, and profitability of the whole industry, all of which could be interrelated within the ecosocial system. To understand the potential weaknesses and challenges behind this complicated ecosystem, real-world data and evidence-based research will be necessary to support the way and the process of how we formulate research questions and reach solutions to those questions.

However, China is a special country, leading in food animal production but lacking infrastructure and capacity-building in aquatic epidemiology. There is only one Chinese government agency at the national level, China Animal Health and Epidemiology Centre (CAHEC), which is responsible for national epidemiological surveillance of livestock diseases, with the exclusion of aquatic animal diseases. Monitoring and surveillance of fish diseases are supervised by multiple government agencies without academically and educationally-competent personnel to conduct epidemiological investigations or research. The Aquatic Disease Surveillance System, a monthly published bulletin, provides briefings on common disease occurrence, but without reliable statistics on numbers of farms or specific risk factors related to fish disease outbreaks.

Aquatic epidemiology is not yet established in the educational curriculum of aquaculture and veterinary colleges at both undergraduate and graduate levels. This leads to the practice of aquatic epidemiology in the prevention and control of fish diseases among provincial and national policy makers in aquatic animal health by individuals who do not yet possess the necessary training, knowledge, and skills.

In China, very few semi-quantitative or quantitative methods have been used for analysis of fish health data collected from farms or from the processing and delivery of live fish. There is a need for the Chinese central government to set up an active consultation on cost sharing, risk factors, and regulation of aquatic animal diseases that occur across the food chain of fish, from pond to table. To prepare for the problem-solving interface, where science and technology can meet science and public policy (Costa-pierce, 2010), the first step is to have a baseline understanding of production problems and how aquatic producers are dealing with them.

Due to the lack of related research, evidence-based research in Chinese aquaculture could begin with the collection and management of field data from different sectors of the warm-water aquaculture industry. This is why we have carried out research on several different topics related to fish producers and fish health management in China.

1.4. Objectives

In the first research chapter, using first-hand survey data, I analyzed the returns and costs of fish farmers in two Provinces representative of Chinese warm-water aquaculture. By using the biosecurity survey data from the same study group in the economics study, I describe how fish farmers think and behave during the health management of pond fish. In the third research chapter based onI then move to the analysis of mortality data based on logbook collected by farm workers hired by a feed company, I apply a time-series regression analysis to explore how the environment, temperature, and handling of fish could be related to the daily mortality of grass carp. In the last research chapter, the logistics of transported live fish to determine variations of mortalities of transported fish upon delivery to wholesale markets. Specific objectives of each study are described as follows.

1.4.1. To estimate farm-level profitability of pond aquaculture

By using survey data from farmers, a returns-costs analysis of freshwater pond aquaculture of yellow catfish in 2 Chinese provinces was done for the following purposes: (1) to estimate returns and costs for yellow catfish aquaculture in China at the farm level, and (2) to provide a spreadsheet-based partial budget analysis tool for farmers to evaluate fish production on their own farms.

1.4.2. To evaluate fish producers' knowledge and biosecurity practices

Based on the survey data on farm-level biosecurity, I quantified behaviors and perceptions of fish farmers culturing yellow catfish in 2 Chinese provinces. This is the first study of biosecurity in small-scale, freshwater pond aquaculture of fish in China.

1.4.3. To explore how stressors affected mortalities of grass carp reared in ponds

I carried out a study to describe mortality patterns of grass carp based on pond-level daily records of a single farm during a production cycle. I used time-series regression methods to explore how predisposing stressors affected the dynamics of daily mort counts of grass carp.

1.4.4. To investigate mortalities of transported fish and related logistical factors

I designed a case study to analyze variation in fish mortality claims and identify potential factors affecting those claims. The specific purposes of the study were to assess whether there was clustering of fish mortality claims among customers over time, and to evaluate customer credibility using a cross-classified model.

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Chapter 2 Farm-level returns and costs of yellow catfish (*Pelteobagrus fulvidraco*) aquaculture in Guangdong and Zhejiang provinces, China

2.1. Abstract

Freshwater aquaculture in China is expanding and intensifying as this country experiences rapid economic growth, and understanding farm-level profitability is necessary if farmers are to make reasonable decisions about their production plans. We conducted a survey of yellow catfish farmers in 2014 in Guangdong and Zhejiang provinces in order to estimate farm-level profitability of pond aquaculture. We selected representative prefectures from the 2 provinces as study areas and used convenience sampling. Eighty-seven farmers were interviewed between April and May 2014 and the questionnaire collected detailed information on: (1) farmers' demographics (age, gender, education, training, and experience); (2) production inputs (land, labor, fingerlings, feed, chemicals, machinery, and other miscellaneous costs); and (3) outputs (weight and revenue of harvested fish). Responses of 61 farmers included in the data analysis were post-stratified into 3 categories of farm size (<1.47 ha, 1.47-3.67 ha, and > 3.67 ha). We calculated production cost components, returns, and returns-costs ratios by farm size in each province. The overall returns-costs ratio was 1.31 in Guangdong and 1.17 in Zhejiang. Farmers in Guangdong invested more in land and machinery and had higher percentages of labor costs and chemical expenditures, but achieved better returns-costs ratios than farmers in Zhejiang. Higher land rent might be associated with greater yields of yellow catfish in Guangdong, which were almost twice those of Zhejiang.

Keywords: Pond aquaculture, farm-level profitability, returns-costs analysis, yellow catfish (*Pelteobagrus fulvidraco),* China

2.2. Introduction

Freshwater finfish aquaculture has expanded in mainland China during the last 20 years, and China now leads globally in production and consumption of farmed freshwater species (Chiu et al., 2013; FAO, 2014). Approximately 70% of freshwater aquaculture production in China is carried out in ponds (Wang et al., 2014), and its development has been influenced by production inputs, including land, or availability of water, fish diseases, and farmers' knowledge and practices (Ahmed et al., 2007a). Thus it is necessary to assess the degree to which their farm-level production inputs are cost-effective for farmers to make decisions on production expansion.

Several Chinese economic studies have evaluated profitability (Chen, 2008; Yuan and Xun, 2009), production function (Chen, 2010), production efficiency (Gao et al., 2012), and cost efficiency (Liu, 2007) of freshwater fish farmers. However, few studies have focused on analyses of returns and costs of freshwater pond aquaculture in China (Chen et al., 1995; Gomiero et al., 1997; Yuan, 2007; Yin et al., 2014). Furthermore, surveyed data were seldom used to examine the extent of input usage in freshwater fish farms in China (Yin et al., 2014). Survey studies are necessary to investigate the current profitability of Chinese freshwater fish farmers.

One native freshwater species that has a strong market demand is yellow catfish (*Pelteobagrus fulvidraco)*, commonly called yellow bonefish by farmers in southern and eastern China (Dong et al., 2011; Liu et al., 2013). Production of yellow catfish has expanded because of widespread availability of fry, innovative feed technology, the species' tolerance for long distance transportation, and its high market value (Tan et al., 2012; Tang et al., 2012). It has now become one of the most important freshwater finfish species in Chinese aquaculture (Wu et al., 2010; Dong et al., 2011). To our knowledge, there are no recently published studies in English using survey data to examine farm-level profitability of pond-based yellow catfish aquaculture in

China. The objective of our study was to estimate returns and costs for yellow catfish aquaculture in China at the farm level.

2.3. Materials and methods

2.3.1. Sampling design for yellow catfish farms

We carried out a survey in 2014 on yellow catfish farms in 2 major freshwater aquaculture provinces, Zhejiang and Guangdong, in China. According to field staff working for one of the largest fish feed-companies in Zhejiang province, Guangdong province has the highest yield of yellow catfish in China, while Zhejiang province is one of the provinces with the longest history of inland freshwater aquaculture.

Foshan prefecture, in Guangdong province, supports 80% of yellow catfish production in that province, and has 3,333-4,000 ha of pond culture of yellow catfish (Su, 2015). We selected Nanhai County in Foshan because it has the largest yellow catfish production among the 5 counties in this prefecture (Yin, 2015). Huzhou is the major prefecture for freshwater aquaculture in Zhejiang province, and has 3,333-4,667 ha of aquaculture ponds (Yin, 2015). Three counties in Huzhou were included in the sampling: Deqing, Nanxun, and Wuxing. Foshan and Huzhou are prefecture-level cities, ranked below a province and above a county in China's administrative structure.

The sample sizes were proportional to the estimated number of yellow catfish farms in the study areas, as shown in Table 1. Estimation of total number of yellow catfish farms in each county was based on the anecdotal notes of aquatic experts, feed-company sales representatives and local fish veterinarians. We have interviewed 44 farmers in Guangdong and 43 in Zhejiang during April and May 2014.

2.3.2. Data collection and data entry

A structured interview (available on request from the authors) was used for data collection in both provinces, and consisted of 3 parts: demographic information, input and output quantities and prices, and biosecurity practices/behavior. The biosecurity behavior data were analyzed in Jia et al. (2015 submitted). Confidentiality protection was first explained to respondents, and interviews were only conducted after farmers' consent. The farmers were sampled conveniently, where we could meet them, either on their own fish farms, at aquatic service stations owned by industry middlemen or at private fish vet clinics. Visits to the farmers were done in the company of field staff working for aquatic feed companies. To facilitate farmers' participation, we only included farmers if feed-company service staff knew them. The interviewer took notes when respondents' answers were outside the range of choices in the questionnaire or when respondents provided additional anecdotal information.

The average yellow catfish production cycle was assumed to be no more than 12 months' duration, with stocking after January 2013 and harvesting before May 2014, which was the last production cycle before the survey. Due to the project budget limitation and rainy season, we only interviewed 44 farmers in Guangdong and 43 in Zhejiang during the study period of April and May 2014 (Table 2.1).

Original survey data were entered into Excel, and each farmer was assigned an identification number to ensure confidentiality. All non-responses were kept blank.

2.3.3. Post-stratification for surveyed farms

Different methods of using covariate variables have been developed for deciding optimum stratification boundaries to reduce the potential error of misclassification (Singh and

Sukhatme, 1969; Singh, 1971; Mahajan and Singh, 2005; Khan et al., 2009; Sebnem, 2011). In this study, farm size was used as the covariate for the post-stratification process. All 87 yellow catfish farms were categorized into 3 classes, using the cube-root cumulative-frequency method, as in Equation 1 (Singh and Mangat, 1996; Shyamalie, 2008). In brief, 2-class post-stratification was found to mix farms of medium and large sizes, and was a less efficient distribution of farms than the 3-class stratification method. Thus, 3-class stratification was applied: Category I (<1.47 ha), Category II (1.47-3.67 ha), and Category III (> 3.67 ha).

$$L_i = y_{i-1} + [(S_k/L - S_{i-1})/\sqrt[3]{f_i}](y_i - y_{i-1}) \qquad \text{Eq.1}$$

Where;

L = No. of strata;

L_i = Upper limit of i th stratum;

y_{i-1} = Lower limit of the class in which L_i lies;

$\sqrt[3]{f_i}$ = Cube root of the frequency of the i th class in which L_i lies;

S_k = Cumulative total of $\sqrt[3]{f_i}$;

S_{i-1} = Cumulative cube root of the frequency of preceding class to the class in which L_i lies;

y_i = Upper limit of the class in which L_i lies;

$y_i - y_{i-1}$= Width of the class in which L_i lies.

2.3.4. Demographics summary and production profiles of study farms

We summarized relevant demographic information of farmers, including gender, age, education background, and aquaculture experience and related professional training. We also conducted descriptive analysis of farm size, characteristics of ponds used for adult fish (pond area, pond size, and pond number), land rent price, harvested biomass, fish yield and feed usage. Apparent feed conversion ratio (AFCR), an appropriate approach for assessing aquafeed utilization efficiencies of carnivorous species (FAO, 1987; FAO, 2002), was estimated by farm size categories.

2.3.5. Returns and costs analyses

The farm-level economics of yellow catfish aquaculture was evaluated using returns and costs analyses (Lipton and Harrell, 1990). In order to increase the accuracy of the production and harvest information, farm data were excluded if harvests were partial, or were full but with incomplete information. I used the following standard terms for costs and returns. All descriptive analyses of survey data were performed using STATA 13 (Stata Corp., College Station, TX, USA).

2.3.5.1. Total fixed costs

Fixed costs are those that are not altered by a change in the level of production (Lipton and Harrell, 1990). Total fixed costs included depreciation of medium- and long-term capital, opportunity cost of using medium- and long-term capital, land rent, land revenue in case of owned land, and wages for permanent labor.

Total medium- and long-term capital referred to the capital used for more than one production cycle. Total medium- and long-term capital included expenditures on machines and other equipment related to aquaculture management, including oxygenators, power generators, harvest nets, feed mixers, and water pumps.

Opportunity cost of using medium- and long-term capital. We assumed that the next best alternative for using medium-and-long term capital was a long-term deposit in the bank. This opportunity cost was calculated as interest forgone for 12 months, using a 60-month deposit rate of 4.75% per annum, as per the Bank of China (Bank of China, 2012).

Depreciation of medium- and long-term capital is the decline over time in the value of capital, primarily due to wear and tear. Based on an assumption of predicted (approximately constant) depreciation, the straight-line method of depreciation was applied, by dividing purchase value at current prices by economic life (in years). The economic life of different farm equipment was assumed, on average, to be: 5 years for oxygenators and feed mixers, 10 years for water pumps, and 15 years for diesel generators and harvest nets.

Land rent was the product of land rent per hectare and farm size. Land in the study areas was generally owned by the villages, and farmers paid rent to the village-level government.

2.3.5.2. Total variable costs (TVC)

Variable inputs are those that change with levels of production (Lipton and Harrell, 1990). The expenditure associated with variable inputs is called short-term capital. TVC is the sum of short-term capital and opportunity cost of using short-term capital.

(I) Total short-term capital

Total short-term capital included expenditures on fingerlings, feed, chemicals, wages of casual/seasonal hired labor, imputed value of part-time family labor, electricity, pond sediment removal, pond disinfection before stocking, and farm equipment repairs.

Feed expenditure referred only to expenditures incurred for supplementary feed, and excluded natural feed found in ponds (i.e. plants). If different supplementary feeds were used on a farm, feed expenditure was calculated as the sum of the expenditures of those different feeds. Single feed cost was calculated as feed price per 1000 kg multiplied by usage.

Chemical expenditure referred to expenditures on all chemicals and biological fertilizers, including antibiotics, anti-parasitics, and other medicines for treatments, water improvement detergents, and other chemotherapeutics related to fish health improvement.

Casual/seasonal hired labor cost included wages for full-time seasonal staff and for casual labor hired only for harvesting fish and seining ponds.

Family labor wages were imputed as total number of months of family members working on a farm, either full-time or part-time, multiplied by average monthly income for farmers in the 2 prefectures. Annual income was reported to be USD 2,823 and USD 3,071 for rural residents in the prefectures of Huzhou (Baidu, 2014) and Foshan (Wu, 2014), respectively.

(II) Opportunity cost of short-term capital

We assumed that the next best alternative for using short-term capital was a short-term deposit in the bank; therefore, opportunity cost was the foregone interest. Opportunity cost of short-term capital was calculated by multiplying total short-term capital for 6 months by a 3-month deposit rate of 2.85% per annum, as per the Bank of China (Bank of China, 2012). It was difficult to calculate the distribution of the use of short-term capital over the year and within a particular month; therefore, we assumed that farmers used equal proportions of short-term capital in the middle of every month.

2.3.5.3. Gross return

Gross return was the total monetary receipt generated by sales of all harvested fish (Yuan, 2007). Farm-level gross return was computed by multiplying total weight of harvested fish (kg) by market price (USD/ kg).

2.3.5.4. Net return

We used 3 different measures for net return in this study, as follows**:**

Net return over total cost (Net return OTC) was computed as gross return minus total costs. In the long run, net return OTC must be positive for the farm to be economically viable.

Net return over variable cost (Net return OVC) was calculated by subtracting total variable costs from gross return. Farmers must cover total variable costs in the short term to remain economically viable, i.e., Net returns OVC should be positive.

Net return to family labor (Net return TFL) was calculated by taking Net return OVC and adding the opportunity costs of family labor, which were the imputed wages. Off-farm work alternatives to aquaculture might be very few or only seasonal available for these fish farmers in the 2 provinces. By assuming that family labor used on the farm was a foregone cost, the imputed wages were approximated as the values of unpaid family labor (Engle, 2010). Except unpaid family labor, other opportunity costs of investing family labor are negligible in this study. In the short term, a net return TFL is an appropriate measure to evaluate the economic viability.

2.3.5.5. Returns-costs ratio and other indicators of farm-level profitability

Calculations for the returns-costs ratio and other economic indicators are outlined in Table 2.8, which were calculated for different sized farms in the 2 provinces.

2.4. Results

Data from 61 of 87 (70.1%) sampled farms (with fully-harvested fish before the end of the survey and with complete production information) were included in the descriptive analysis. Demographic information was summarized by province. Production and returns-costs analyses were evaluated and reported, based on post-stratified farm-size groups for each province.

2.4.1. Demographic information

Among the 61 yellow catfish farmers interviewed, there was only one female respondent in Guangdong, and no female respondents in Zhejiang (Table 2.2). The mean ages of farmers were 53 (range, 30-65) and 50 (range, 25-65) for Guangdong and Zhejiang, respectively. Forty-four percent of farmers in Guangdong were in the age group ranging from 55- 65 years, while 71% of farmers in Zhejiang were within the age group of 41-55 years.

Most yellow catfish farmers in both provinces had elementary school education. In Guangdong, 12% of farmers had not completed elementary school. A slightly higher percentage (26%) of middle school was reported in Zhejiang province. One farmer (4%) in Zhejiang had a college education.

Aquaculture experience was defined as the number of years that a farmer had been involved in any form of aquaculture production, and was not confined to yellow catfish culture, which was encouraged in the study areas starting in about 2002. Prior to 2002, most farmers in both provinces were involved in carp production. In this study, farmers in Guangdong and Zhejiang averaged 16 to 18 years' experience in the industry (Table 2.2). Interestingly, 30% of respondents in Guangdong had 6-10 years of experience, and might not have had any experience with freshwater aquaculture before they started growing yellow catfish. The highest percentage (32%) of farmers reporting the most experience (21-25 years) was in Zhejiang. For each province, almost 50% of farmers had more than 15 years of experience. Only 6% and 22% of farmers in Guangdong and Zhejiang provinces, respectively, reported having formal aquaculture training.

2.4.2. Production systems

2.4.2.1. Farm size, biomass harvested and yellow catfish production

Mean farm sizes were 0.7 ha and 2.1 ha for Guangdong and Zhejiang provinces, respectively (Table 2.3). Most farms in Guangdong were small, belonging to category I (<1.47 ha), which decreased the mean value of farm size for this province. In contrast, almost 50% of farms in Zhejiang belonged to the 2 upper strata of farm sizes (1.47 to 3.67 ha and ≥3.67 ha).

Farmers in Zhejiang tended to have larger single pond sizes and larger farm sizes, total area of ponds per farm and total number of adult ponds per farm (Table 2.3). Farms in Guangdong had, on average, smaller fish ponds (0.36 ha/pond) than those in Zhejiang (0.65 ha/pond). The biggest average pond size of 0.82 ha was in the category III farms in Zhejiang, which was almost twice the average pond size of farms in Guangdong (Table 2.3). The maximum total number of ponds (14 ponds per farm) was also reported from the category III farms in Zhejiang, compared with at most 4 ponds per farm in Guangdong. The average land rent per hectare in Guangdong (USD

6,034) was almost 3-fold higher than in Zhejiang (USD 2,109), which reflected a geographical difference in land costs and land allocations between and within provinces (Table 2.3).

The total biomass harvested per farm in category I was 16.1 metric tons and 13.1 metric tons for Guangdong and Zhejiang, respectively (Table 2.3). Farms in Guangdong had higher average biomass harvested per hectare (27.6 metric tons/ha) than those in Zhejiang (16.3 metric tons/ ha). The average biomass per unit of land was similar among farms in the 2 farm-size categories in Guangdong. However, this was not the case in Zhejiang, where category III farms produced on average 18.4 metric tons of fish per hectare, while 16.7 metric tons per hectare was reported from category I, and 14.8 metric tons per hectare from category II farms in that province.

The average yield of fish in Guangdong (25.68 metric tons/ha) was more than twice that of Zhejiang (14.59 metric tons/ha) (Table 2.4). The lowest yellow catfish yield (7.75 metric tons/ha) was in category II farms in Zhejiang, among which the same type of farms had mixed species farming and polyculture of yellow catfish (Table 2.4). Mixed species farming of yellow catfish occurs when a farmer produces yellow catfish and other freshwater fish in different ponds on the same farm. Polyculture is when yellow catfish are raised with other species in the same pond. Fish farmers in Zhejiang province had a higher proportion of culture combinations, with 61% practicing monoculture and 39% farming polyculture or mixed species. White carp (*Erythroculter ilishaeformis*) was the species usually stocked with yellow catfish in polyculture operations. Black carp (*Mylopharyngodon piceus*) and yellow catfish (*Pelteobagrus fulvidraco)* were cultured in different ponds from yellow catfish farms in category II farms in Zhejiang Province (Table 2.4). However, in Guangdong province, most farmers (97%) kept yellow catfish in monoculture on the same farm, and only 3% of farmers practiced mixed-species farming and there was negligible yield information for the different species in polyculture.

2.4.2.2. Feed brand choices, feed usage, and crude protein input

Two different types of domestic fish feed companies (local and non-local) supply farms. We differentiated them based on production scales and marketing strategies. Interestingly, 88% of farmers in Guangdong chose non-local companies, while in Zhejiang almost equal percentages of farmers chose non-local (57%) and local companies (43%). Most category I farms in Guangdong chose non-local companies, while among farms in the same category in Zhejiang, the number of farms choosing non-local feed companies was 1.5 times greater than those choosing local feed companies.

In this study, the feed input for yellow catfish farms in Guangdong was 44.95 metric tons per hectare, while for farms in Zhejiang it was 22.59 metric tons per hectare (Table 2.5). Crude protein usage in the 2 provinces showed a similar difference. Farms in Guangdong had an overall higher estimated apparent feed conversion ratio (AFCR) (Table 2.5). The average AFCRs of farms in Guangdong were: 1.83 for category I, and 1.55 for category II. In contrast, there was no apparent variation of AFCRs among different types of farms in Zhejiang.

2.4.3. Returns and cost analysis

2.4.3.1. Capital requirements and costs

Costs varied among different types of farms in the 2 provinces; for example, total fixed costs ranged from 4.6% to 10.1%, and total variable costs ranged from 90.2% to 95.3% (Tables 2.6-7). Extreme values of the cost components were found in category II farms in Guangdong and category III farms in Zhejiang. Farmers in Guangdong invested less short-term capital than those in Zhejiang while, in contrast, the amount of medium- and long-term capital invested in Guangdong was higher than in Zhejiang.

(I) Total fixed cost

Medium- and long-term capital in Guangdong (USD 2,492) was about 2-fold higher than in Zhejiang (USD 1,401) (Table 2.6). Farmers in Zhejiang invested 91.1% of their medium- and long-term capital on oxygenators (Table 2.6), while machinery investments by farmers in Guangdong included other aquaculture equipment, i.e. diesel generators, harvest nets, feed mixers, and water pumps.

Differences in total fixed cost components, particularly land rent percentages, existed among farms in the 2 provinces (Table 2.6), while the overall percentage of land cost of farms in Guangdong was 50% higher than in Zhejiang. Farmers in Zhejiang reported reduced production costs by not investing in aquaculture equipment, such as harvest nets, feed mixers, and water pumps. Furthermore, the percentage differences of depreciation of medium- and long-term capital between the 2 provinces were directly related to total medium- and long-term capital investments. Farmers in Categories I and II in Guangdong reported larger investment in aquatic machinery than farms in the same categories in Zhejiang.

(II) Total variable cost

Total variable cost as a percentage of total cost was 94.8% in Zhejiang and 91.4% in Guangdong (Table 2.7). Compared with medium- and long-term capital requirements, the short-term capital requirement was responsible for the majority of total costs, 90.7% in Guangdong and 93.5% in Zhejiang. Among all components of total short-term capital of farms in both provinces, feed cost was the highest of all single item costs listed, ranging from 69.1% to 74.6%. The percentages of the short-term capital from feed, fingerlings, and labor were very similar. However, the percentage for fingerlings in Zhejiang was almost twice that of Guangdong. Cost of electricity was much higher in Guangdong, as a percentage of total variable costs, than in Zhejiang. Finally, farmers in both provinces shared similar percentages for chemical expenditures, hired labor use, and family labor costs.

2.4.3.2. Returns and returns-costs ratios

The average gross return in Zhejiang was USD 91,698, compared with USD 70,716 in Guangdong, but this was skewed by the large Category III farms in Zhejiang (Table 2.8). On average, farms in Category I in Guangdong achieved a gross return of USD 62,243, while the gross return for the same size category in Zhejiang was USD 43,843 (Table 2.8).

Farmers in Guangdong had a higher overall average value for all 3 measures of net return than those in Zhejiang (Table 2.8). These were: net return over total costs, net return over total variable costs, and net return to family labor.

Overall returns-costs ratios for Guangdong and Zhejiang were 1.31 and 1.17, respectively, while the estimated average cost per harvest biomass (USD/kg) was numerically higher in Guangdong (Table 2.8). The average cost per hectare in Guangdong (USD 77,217) was more than twice than that in Zhejiang (USD 37,164) (Table 2.8). After we calculated net return per harvest fish weight, we also found a difference of net return per kg of USD 0.44 between farms in Guangdong and Zhejiang (Table 2.8). The net return per ha of farms in Guangdong was also numerically higher than those in Zhejiang, with farmers in Guangdong receiving more than twice the net return per hectare of farmers in Zhejiang.

2.5. Discussion

Our returns and costs analyses showed that the lowest net returns per kg of harvested fish were obtained for farms of category III in Zhejiang and the highest were from in category II farms in Guangdong. Although total costs per kg were much lower, on average, for similar sized farms in Zhejiang, gross returns were higher on farms in Guangdong province. In general, farmers in Guangdong had higher net returns than those in similar size categories in Zhejiang. When we compared category I farms, net return in Guangdong was two-fold higher than that of Zhejiang. Category II farms in Guangdong had the highest net return per harvest biomass (USD 1.34/kg). If the family labor was excluded from short-term capital, farmers in Guangdong realized net returns almost 2.5 times higher than farmers in Zhejiang.

One possible explanation why farmers in Guangdong had a higher gross return than similar sized farms in Zhejiang was that they raised fish at much higher densities. High-density stocking may be used because land in that province was more expensive than in Zhejiang. Farmers in Guangzhou likely chose small farm sizes to avoid the high cost of land rent, but compensated by raising more fish per hectare of land. This trend in their business strategy is consistent with the negative relationship reported by Chen et al. (2011) between farm size and profitability.

We speculated that the success of “extremely” high density stocking of yellow catfish in Guangdong might be related to favorable weather conditions, the availability of genetic improvement breeding programs, land price pressures, and market demand. Guangdong is located close to the tropics, where the production cycle is shorter for yellow catfish, as it can be cultured almost year round, compared to Zhejiang. During our study period, high-density farming of this species appeared to benefit farmers in Guangdong, but it might also be a higher risk. In outbreaks of infectious disease, the higher the density of fish in a pond the higher the mortalities (Plumb, 1999), which could reduce gross return significantly.

The mean market price of yellow catfish for farmers in Guangdong was USD 3.94/kg compared with USD 3.47/kg in Zhejiang. Thus, in addition to high yield, farmers in Guangdong also were paid more for their fish. In China, live fish are preferred by consumers. The development of aquatic logistics companies that transport fish over long distances has made it possible to sell live freshwater fish in almost every provincial capital city in China, even though the transportation distance to northern and western China markets is more than 3000 km from Guangdong. This has opened significant markets for fish farmed in Guangdong. Seventy percent of yellow catfish in Guangdong were reported as transported to other provinces (according to anecdotal notes). In contrast, fish transport companies are not as well established in Zhejiang, and the markets for farmers in this province are limited to local and adjacent provinces.

Farmers in Zhejiang tended to practice multi-age stocking, polyculture and mixed species rearing while yellow catfish were mostly farmed in monoculture systems in Guangdong. Because we focused more on returns from yellow catfish, we may have underestimated the gross return from farms in Zhejiang relative to those in Guangdong, but the missing returns from other species is unlikely to make up the difference in gross return found on average between the 2 provinces. We could not include the returns from these other fish species because we did not have their production cost data.

Costs of producing fish in Zhejiang were lower than costs in Guangdong because farmers in Zhejiang stocked ponds in low densities. In general, farming at higher densities increases costs such as electricity and use of oxygenators, which is the first limiting factor for intensive aquaculture operations (Yu et al., 2008), and is what we found in our study. Interestingly, the cost of fingerlings and feed per production kg was lower in Guangdong than in Zhejiang and, despite the fact that feed costs are usually the highest variable costs, this difference did not offset the increased price of electricity and supplemental oxygen.

Feed is one of the most important factors influencing total cost. We found variation in farmers' decision-making in 2 aspects of feed input influencing cost of feed to be used for the same yield of fish: source of feed (local or non-local) and type of feed (floating or pellet). In this study, price was a major factor influencing farmers' choice of feed company. Most farmers in Guangdong chose non-local feed companies, while farmers in Zhejiang did not have an apparent preference for local or non-local sources. Expansion of the aquatic feed industry has been one of the major driving forces for aquaculture development in China. Feed price from non-local companies is usually higher. Non-local, trans-provincial companies use the branding of their products to improve their sales through large sales forces and technology service teams, which have larger organizational structures and marketing capacities than small-scale local companies. Both types of companies need middlemen to assist with sales and service. Feed payment methods might also influence feed prices. Farmers normally paid lower prices if they were able to make prepayments of 5-10% of their estimated annual feed cost, before stocking, or cash payments after shipment of feed to their farms (personal communication during interviews). According to anecdotal reports from fish health service personnel, other factors related to this decision-making process might include feedback from other farmers, and recommendations from middlemen.

We expected a low AFCR with more expensive feed. Aside from category I farms in Guangdong, it was surprising how similar the AFCRs were between farms in the 2 provinces despite the higher feed cost in Zhejiang. Category I farms in Guangdong had higher AFCR than all other farms in the study, but this might be related to use of cheaper feed. All yellow catfish farmers in Zhejiang used only floating feed, with a range of price USD 1370 -1580/metric ton, while to cut feed costs, many farmers in Guangdong used floating feed combined with powder feed, which was called Hong Kong catfish (*Clarias fuscus*) feed (about USD 483/metric ton). Other factors potentially affecting the net returns of farms in Zhejiang included price of fish at harvest, fingerling cost, and low fish yield per hectare.

Yellow catfish is an indigenous catfish species in China, and aquaculture of indigenous catfish species is important to local aquaculture sectors in other Asian countries, such as farming of stripped catfish (*Pangasianodon hypophthalmus*) in Thailand, Vietnam and Bangladesh. Similar to findings of this study, previous economic studies have shown that intensive aquaculture of stripped catfish is profitable in those countries (FAO, 2010; Nguyen, 2013). Relevant studies in this region indicated that return-cost ratios of stripped catfish production in Bangladesh were 1.73 from field studies in rural areas (Ahmed and Hasan, 2007b) and 1.08-1.36 by experimental studies (Khan et al., 2009b). Because feed cost is the highest single cost component, collective evidence indicated low FCR might increase the net profit of stripped catfish aquaculture (Da et al., 2013).

Although our study identified some differences in costs and returns between similar sized farms in 2 different provinces in China, interpretation of the information collected should be done with caution as it was collected using farm surveys and not financial statements. Farm surveys are acknowledged as an indispensable tool for the estimation of costs of agriculture production (Ronzon et al., 2014). However, there are limitations to collecting financial information in this manner. It can be biased by the farmer intentionally or un-intentionally, and often prices and costs are only estimated and could be erroneous. This study had to omit several farms from the returns and costs analyses because of incomplete data, which was predominantly due to a lack of detailed record keeping of production and harvest data. As fish farms in China become larger their record-keeping improves out of necessity and, therefore, we likely biased our sampling towards more organized farmers and larger farms.

We also likely had sampling bias because we used convenience and opportunistic sampling, as there is no official list of yellow catfish farms in China from which to establish a sample frame. Further, we had limited resources for visiting farms. We received assistance from local staff affiliated with aquatic feed companies in both locations, who connected us with intermediate traders to recruit farmers to participate in the interviews. The inclusion of farmers was likely dependent on their relationships with these feed representatives. There were several farmers who had not started or completed a harvest during our interview window, so they could not provide complete information on returns and costs, and were excluded. Incomplete questionnaires were also an issue as some individuals did not want to provide economic data to us.

2.6. Conclusions

Our findings indicated that returns-costs ratios were slightly higher in Guangdong than in Zhejiang probably due to the higher productivity and market price. Farmers in Guangdong spent more money on fixed and variable costs, i.e. land and electricity, but feed and land rental costs were the major costs to decrease net returns in both provinces. Market price strongly affected net returns in the yellow catfish aquaculture in both provinces..

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Table 2.1 Source population and sample size of yellow catfish farms investigated in Guangdong and Zhejiang provinces in 2014.

Province	Prefecture	County	Estimated number of farms in each county	Total number of farms in sample frame	Total number of farmers interviewed	Total number of farms analyzed
Guangdong	Foshan	Nanhai	3,000	50	44	33
Zhejiang	Huzhou	Deqing	800	15	10	8
		Nanxun	1,000	20	19	10
		Wuxing	1,000	20	14	10

Table 2.2 Demographic information of yellow catfish farmers in in Guangdong and Zhejiang provinces in 2014.

	Guangdong		Zhejiang	
Variables	Response rate (%)	Number (%)	Response rate (%)	Number (%)
Gender	100	33 (100)	100	28 (100)
Female		1 (3)		0
Male		32 (97)		28 (100)
Age (years)	94	32	100	28
25-40		4 (13)		2 (7)
41-55		13 (41)		20 (71)
56-65		14 (44)		6 (21)
Education	100	33	96	27
Below elementary		4 (12)		0
Elementary		21 (64)		18 (67)
Middle school		5 (15)		7 (26)
High school		3 (9)		1 (4)
College		0		1 (4)
Aquaculture experience (years)	100	33	100	28
1-5		4 (12)		2(7)
6-10		10 (30)		5(18)
11-15		3 (9)		5 (18)
16-20		8 (24)		7 (25)
21-35		8 (24)		9 (32)
Exposure to aquaculture training	100	33	96	27
With training		2 (6)		6 (22)
Without training		31 (94)		21 (78)

Table 2.3 Farm size, pond area, pond size, pond number, land rent per hectare and harvested fish of yellow catfish farms in Guangdong and Zhejiang provinces in 2014. n = number of farmers.

Variables	Guangdong			Zhejiang			
	Category I[a] (n=31)	Category II[a] (n=2)	Overall (n=33)	Category I[a] (n=15)	Category II[a] (n=9)	Category III[a] (n=4)	Overall (n=28)
Mean area of farm (ha)	0.63	2.50	0.74	0.74	2.01	7.18	2.07
Mean area of adult pond per farm (ha)	0.55	1.93	0.64	0.73	1.79	5.94	1.81
Mean water surface area per adult pond studied (ha)	0.35	0.48	0.36	0.55	0.76	0.82	0.65
Mean land rent per hectare (USD/ha)	6,040	5,927	6,034	2,232	2,105	1,730	2,119
Median and range of total number of adult ponds studied per farm	1 (1- 3)	4 (4-4)	4 (1- 4)	1(1- 4)	2 (1- 6)	6 (4-14)	2 (1-14)
Mean total biomass harvested per farm (ton)	16.1	49.3	18.1	13.1	23.9	96.6	28.5
Mean biomass per hectare harvested (ton/ha)	27.6	27.2	27.6	16.7	14.8	18.4	16.3
Mean fish production [b] (kg)	14,723	48,650	18,545	12,098	20,497	86,099	25,369

[a] Three-class stratification of yellow catfish farms was applied: category I (<1.47 ha), category II (1.47 - 3.67 ha), and category III (> 3.67 ha). [b] Fish production is the net biomass gain of the production cycle (total biomass harvested minus total initial biomass).

Table 2.4 Fish yield (1000 kg/ha) of yellow catfish farms in Guangdong and Zhejiang provinces in 2014. n = number of farmers.

Variables	Guangdong			Zhejiang			
	Category I [a] (n=31)	Category II [a] (n=2)	Overall (n=33)	Category I [a] (n=15)	Category II [a] (n=9)	Category III [a] (n=4)	Overall (n=28)
Average yield of all fish	25.65	26.87	25.68	15.25	12.70	16.40	14.59
Yield of yellow catfish	25.65	26.87	25.68	13.99	7.75	13.92	11.98
Yield of black carp					1.43		0.46
Yield of white carp				1.37	3.09	2.48	2.08
Yield of other fish					0.45		0.14

[a] Three-class stratification of yellow catfish farms was applied: category I (<1.47 ha), category II (1.47 - 3.67 ha), and category III (> 3.67 ha).

Table 2.5 Commercial feed and crude protein input of yellow catfish farms in Guangdong and Zhejiang provinces in 2014. n = number of farmers.

	Guangdong			Zhejiang			
Variables	Category I [a] (n=31)	Category II [a] (n=2)	Overall (n=33)	Category I [a] (n=15)	Category II [a] (n=9)	Category III [a] (n=4)	Overall (n=28)
Commercial feed							
Total commercial feed usage (1000 kg)	25.96	73.50	28.84	19.19	31.20	119.75	37.41
Commercial feed usage per area (1000 kg/ha)	45.29	39.78	44.95	24.60	18.48	24.36	22.59
Crude protein input							
Total crude protein input (1000 kg)	10.05	28.73	11.18	7.69	12.62	33.41	12.95
Crude protein per area (1000 kg/ha)	17.34	15.62	17.23	9.86	7.43	7.61	8.76
Apparent feed conversion ratio							
AFCR (Feed usage/fish production)[b]	1.83	1.55	1.81	1.56	1.57	1.42	1.55

[a] Three-class stratification of yellow catfish farms was applied: category I (<1.47 ha), category II (1.47 - 3.67 ha), and category III (> 3.67 ha).
[b] Fish production is the net biomass gain of the production cycle (equal to total biomass harvested minus total initial biomass).

Table 2.6 Capital requirements of yellow catfish farms in Guangdong and Zhejiang provinces in 2014. n = number of farmers (currency in USD).

	Guangdong						Zhejiang							
	Category I [a] (n=31)		Category II [a] (n=2)		Overall (n=33)		Category I [a] (n=15)		Category II [a] (n=9)		Category III [a] (n=4)		Overall (n=28)	
Variables	USD	%	USD	%	USD	%	USD	%	USD	%	USD	%	USD	%
I. Total medium- and long-term capital	2,244	100	5,516	100	2,492	100	688	100	1,037	100	4,906	100	1,401	100
i. Oxygenator	1,131	50.4	3,339	61	1,265	50.8	623	90.6	951	91.7	4,462	90.9	1,276	91.1
ii. Diesel generator	312	13.9	645	12	332	13.3	65	9.4	86	8.3	363	7.4	114	8.1
iii. Harvest net	47	2.1			44	1.8					81	1.7	11	0.8
iv. Feed mixer	83	3.7			78	3.1								
v. Water pump	671	29.9	1,532	28	773	31								
II. Total short-term capital	44,084	100	120,411	100	48,710	100	33,671	100	59,254	100	250,980	100	72,938	100
i. Fingerling expenditure	3,457	7.8	5,806	4.8	3,599	7.3	3,592	10.7	8,043	13.6	41,352	16.5	10,417	14.3
ii. Feed expenditure	31,857	72.3	83,226	69.1	34,970	71.8	25,110	74.6	42,228	71.3	186,786	74.4	53,709	73.6
iii. Chemical expenditure	1,541	3.5	7,742	6.4	1,917	3.9	773	2.3	2,428	4.1	4,726	1.9	1,870	2.6
iv. Hired labor wages	827	1.9	2,258	1.9	914	1.9	419	1.2	735	1.2	4,839	1.9	1,152	1.6
v. Imputed value of family labor	3,278	7.4	3,999	3.4	3,322	6.8	3,072	9.1	3,413	5.8	4,607	1.8	3,401	4.7
vi. Other short-term expenditure	3,124	7.1	17,379	14.4	3,988	8.2	705	2.1	2,407	4	8,669	3.5	2,390	3.3
(1) Electricity	3,089	7.0	17,379	14.4	3,956	8.1	694	2.06	1,864	3.1	7,822	3.17	2,088	2.9
(2) Pond bottom mud removal	34	0.1			32				502	0.83			162	0.2
(3) Pond bottom disinfection							12	0.04	41	0.07	565	0.22	100	0.1
(4) Farm equipment amending											282	0.11	40	0.1

[a] Three-class stratification of yellow catfish farms was applied: category I (<1.47 ha), category II (1.47 - 3.67 ha), and category III (> 3.67 ha).

Table 2.7 Costs associated with yellow catfish farming in two Guangdong and Zhejiang provinces in 2014. n = number of farmers (currency in USD).

	Guangdong						Zhejiang							
	Category I [a] (n=31)		Category II [a] (n=2)		Overall (n=33)		Category I [a] (n=15)		Category II [a] (n=9)		Category III [a] (n=4)		Overall (n=28)	
Variables	USD	%	USD	%	USD	%	USD	%	USD	%	USD	%	USD	%
I. Total fixed cost	4,064	8.3	13,663	10.1	4,648	8.6	1,832	5.1	4,143	6.4	12,279	4.6	4,067	5.2
i. Opportunity cost of using medium- and long-term capital	107	0.2	262	0.2	118	0.2	33	0.1	49	0.1	233	0.1	67	0.1
ii. Depreciation on medium- and long-term capital	221	0.5	530	0.4	240	0.4	67	0.2	101	0.2	476	0.2	136	0.2
iii. Land rent	3,736	7.6	12,871	9.5	4,290	8	1,732	4.8	3,993	6.1	11,570	4.3	3,864	4.9
II. Total variable cost	44,712	91.7	122,127	89.9	49,404	91.4	34,151	94.9	60,099	93.6	254,556	95.4	73,977	94.8
i. Total short-term capital	44,084	90.4	120,411	88.7	48,710	90.1	33,671	93.6	59,254	92.3	250,980	94.1	72,938	93.5
ii. Opportunity cost using short-term capital	628	1.3	1,716	1.3	694	1.3	480	1.3	845	1.3	3,576	1.3	1,039	1.3
III. Total cost (I + II)	48,776	100	135,790	100	54,052	100	35,982	100	64,242	100	266,835	100	78,044	100

[a] Three-class stratification of yellow catfish farms was applied: category I (<1.47 ha), category II (1.47-3.67 ha), and category III (> 3.67 ha).

Table 2.8 Returns from yellow catfish farms in Guangdong and Zhejiang provinces in 2014. n = number of farmers (currency in USD).

Variables	Guangdong			Zhejiang			
	Category I [a] (n=31)	Category II [a] (n=2)	Overall (n=33)	Category I [a] (n=15)	Category II [a] (n=9)	Category III [a] (n=4)	Overall (n=28)
I. Gross return	62,243	202,048	70,716	43,843	75,044	308,623	91,698
II. Net return (gross return - total costs)	13,467	66,258	16,664	7,861	10,802	41,788	13,654
III. Net return over variable cost (gross returns - total variable costs)	17,531	79,922	21,312	9,692	14,945	54,067	17,720
IV. Net return to family labor (III + imputed value of family labor[b])	20,809	83,921	24,634	12,764	18,358	58,674	21,121
V. Returns-costs ratio (Gross returns /total costs)	1.28	1.49	1.31	1.22	1.17	1.16	1.17
VI. Other indicators of profitability							
i. Average cost per kg [c] (Total cost / total harvested fish biomass)	3.03	2.75	2.99	2.75	2.69	2.76	2.74
ii. Average cost per ha (Total fixed cost + total variable cost) / total farm size	77,421	54,316	73,043	48,625	31,961	37,164	37,703
iii. Gross return per kg [c] (Gross return / total weight of harvested fish biomass)	3.87	4.1	3.91	3.35	3.14	3.19	3.22
iv. Gross return per ha (Gross return / total farm size)	98,789	80,819	95,562	59,247	37,335	42,984	44,299
v. Net return per kg [c] (Net return OVC / total harvested fish biomass)	0.84	1.34	0.92	0.6	0.45	0.43	0.48
vi. Net return per ha (Net return OVC / total farm size)	21,377	26,503	22,519	10,623	5,374	5,820	6,596
vii. Net return to family labor per ha (Net return to family labor / total farm size)	33,031	33,568	33,290	17,248	9,133	8,172	10,203

[a] Three-class stratification of yellow catfish farms was applied: category I (<1.47 ha), category II (1.47-3.67 ha), and category III (> 3.67 ha).
[b] Family labor was the Variable v. in Section II.Total short-term capital of Table 2.6.
[c] Fish harvest biomass as denominators of i, iii and v in the returns-costs analyses was from Table 2.3.

Chapter 3 Biosecurity knowledge, attitudes and practices of farmers culturing yellow catfish (*Pelteobagrus fulvidraco*) in Guangdong and Zhejiang provinces, China

3.1. Abstract

Biosecurity has been identified as a core concept for Asian freshwater aquaculture, for purposes of food safety, aquaculture sustainability, and trade. To my knowledge, no published studies have evaluated producers' knowledge of biosecurity and biosecurity practices in Chinese aquaculture. I carried out a questionnaire-based knowledge, attitudes, and practices (KAP) study of farmers culturing yellow catfish in Guangdong and Zhejiang provinces in China. Yellow catfish is a freshwater fish with high market value, and is mostly cultured in ponds on small-scale farms. The purpose of this survey was to evaluate biosecurity practices currently applied on those freshwater farms. Convenience sampling was used and 87 farmers were enrolled for interviews, and responses from 50 farmers were included in the data analysis. Demographics of participating farmers and their responses to each question were described. A KAP index was developed to categorize and subcategorize KAP questions, and assigned scores to farmers' original answers to each question. Every farmer was evaluated with both specific scores for their farm-level biosecurity and with score boundaries of "Good" (≥75%), "Fair" (50% to 75%), and "Poor" (<50%). Results of this study indicated that: (1) farmers' basic knowledge of pathogens was good, but their understanding of pathogen transmission was limited; (2) overall scores for attitude towards biosecurity were slightly higher than practices scores, although they were still relatively low; and (3) all farmers were classified as poor or fair for their compliance with overall farm-level biosecurity measures. The findings of this study showed that: farmers' practices were mostly not in compliance with the principles of biosecurity, especially in regards to prevention of pathogen introduction and spread; and the inconsistencies between practices and attitudes indicated that it may be feasible to improve farmer's implementation of aquatic biosecurity at the farm level. Biosecurity concepts evaluated in the study were cited from salmonid aquaculture and their relevance to biosecurity measures of catfish aquaculture in China should be explored in future research. I recommend that current farm-level biosecurity practices in finfish aquaculture in China be improved through investment in education and training, in collaboration with academia and industry partners.

Key words: Biosecurity, KAP survey, freshwater, pond aquaculture, yellow catfish (*Pelteobagrus fulvidraco*), China

3.2. Introduction

Emerging infectious diseases in farmed freshwater finfish often cause devastating socio-economic losses during outbreaks, with on-going mitigation costs due to the persistence of pathogens in freshwater systems (Labrie et al., 2008; Walker et al., 2010; Okamura and Feist, 2011). Disease outbreaks in modern aquaculture production are regarded as bottlenecks for sustainability of freshwater aquaculture (Bostock et al., 2010; Mylonas and Robles, 2014) and a threat to global food security (Prasad, 2010; Santander, 2012; Stentiford et al., 2012).

Modern intensive fish aquaculture facilities share the common characteristics of high fish densities and associated environmental impacts (Diana et al., 2013). In these aquaculture systems, environmental stress has been identified as a major factor in triggering the breakdown of population immunity against infectious disease and has led to opportunities for endemic pathogens to flourish (Sung et al., 2011; Stendera et al., 2012). Furthermore, many infectious diseases of farmed fish are introduced and spread by movements of infected fish or contaminated equipment (Federation of Veterinarians of Europe, 2014). In contrast to terrestrial animal pathogens, aquatic pathogens are more difficult to exclude from production systems due to water connectivity (Peeler and Otte, 2014). There is an ongoing need to reduce vulnerabilities of freshwater aquaculture systems to microbes through disease control strategies (Miranda et al., 2013). As production scales have grown and intensified, disease has become one of the most important economic threats for fish farmers in China (Li et al., 2011).

Biosecurity principles for prevention of aquatic animal diseases are modifications of those used in terrestrial animal health management (Bebak, 1998). Since their initial formal application in Norway in the 1970s, biosecurity principles have been shown to be economically and ecologically efficient in minimizing disease risk in aquaculture production (Delabbio et al., 2005; Chinabut and Puttinaowarat, 2005; Skall and Olesen, 2011; Werkman et al., 2011; Mony and Hasan, 2012). Based on epidemiological evidence of risk factors for fish diseases, aquatic biosecurity practices refer to the collective actions taken at international, national, local, and farm levels to reduce risks of introduction of microbes and to reduce their spread between individuals, populations, farms, or ecosystems (Hardy-Smith, 2006; Mohan et al., 2008; Oidtmann et al., 2011). For purposes of food safety, aquaculture sustainability, and trade, biosecurity has been identified as a core concept for disease prevention in Asian freshwater aquaculture (Bondad-Reantaso, 2005). To our knowledge, there are no published studies that have evaluated producers' biosecurity practices or knowledge of biosecurity at the farm level in Chinese aquaculture.

Quantification of freshwater fish farmers' understanding of biosecurity practices could inform educational and training programs to prevent and control fish diseases. The Knowledge, Attitudes, and Practices (KAP) survey, also known as Knowledge, Attitudes, Behavior, and Practice (KABP) survey, was originally developed in the 1950's for family planning in the western world (Ratcliffe, 1976). In this study, our objective was to describe farmers' biosecurity activities and explore potential drivers behind disease control strategies for aquatic animal pathogens (Delabbio, 2006). To achieve our objective, we conducted a survey of yellow catfish farmers in Zhejiang and Guangdong provinces in China in 2014.

3.3. Materials and methods

3.3.1. Sampling design for yellow catfish farms

Study sites, source and target populations, and sample size are described in Chapter 2, and are summarized in Table 3.1.

3.3.2. Questionnaire design

The questionnaire was modified from an unpublished pilot survey designed to measure biosecurity of carp farms in Hubei province of China in 2013. We designed the questionnaire in English, and translated it into Chinese. To improve response rates, all farmers were visited and paper-and-pencil interviews were conducted (Kaliyaperumal, 2004).

We considered recommendations for a paper-based questionnaire design in terms of format, order of questions, language, and page design (Fanning, 2005; Barua, 2013). An economics survey followed the KAP questions at each interview, and those results are presented in Jia et al. (2016). KAP and economics questions shared demographic information from respondents, including residential addresses, age, gender, academic education, experience, and training in aquaculture.

The structured survey was composed of 75 close-ended questions, with either single or multiple answers. The interviewer took necessary notes when respondents' answers were outside the range of choices in the questionnaire, or when respondents provided additional anecdotal information. We also designed a few questions with similar topics to test reproducibility and internal consistency of the questionnaire (de Vet, et al., 2006; Peer and Gamliel, 2011). Content and format of questions are summarized in the following 3 sections, with specific objectives:

(1) Knowledge: These questions tested whether farmers had basic knowledge of aquatic diseases, including causative agents of fish diseases and potential modes of transmission. Farmers' answers to these questions were "yes", "no", or "not sure".

(2) Attitudes towards biosecurity measures: Respondents could answer these questions on a 4-point scale, as "very useful", "moderately useful", "not useful", or "not sure". Attitudes questions were all asked after practices questions to avoid respondent bias caused by leading questions (Swann et al. 1982).

(3) Practices: Questions in this section determined whether farmers' behaviors were in compliance with biosecurity concepts. Some biosecurity measures in the attitudes questions were repeated in this section. Questions about management practices were also included to describe what farmers did to prevent disease related loss of yellow catfish. Choices of answers in this section were "often-seldom-never" or "yes-no-not always". Some questions had multiple answers, where farmers were asked about disposal of fish carcasses, water quality monitoring, and pond preparation before stocking (if a disease outbreak occurred in previous production cycle).

3.3.3. Data collection

I used convenience sampling to include yellow catfish farmers, based on their relationships with feed company service staff. Interviews were conducted on premises where we could meet farmers, including farms, service stations owned by industry middle-men, and private fish vet clinics.

Confidentiality protection was first explained to respondents, and interviews were only conducted with farmers' informal consent. All interviews were assisted by field staff working for aquatic feed companies. In Guangdong province, translation of the questionnaire into Cantonese was necessary when farmers couldn’t understand Mandarin.

A questionnaire pretest was done in each province to ensure appropriate wording of questions and minimize information bias, which might originate from jargon, or other problems which might impair farmers’ proper understanding of questions (Terwee et al., 2007).

3.3.4. Data entry and analysis

3.3.4.1. Data entry

Original survey data were entered into Excel (Microsoft, Redmond, WA, USA), and each farmer was assigned an identification number to ensure confidentiality. All non-responses were kept blank.

3.3.4.2. KAP question categorization

Categorization of knowledge questions was based on risk factors that could facilitate introduction of infectious pathogens into fish farms (external biosecurity) and spread within facilities or to other fish farms (internal biosecurity) (Arthur et al., 2008; Oidtmann et al., 2014). External biosecurity measures referred to practices that may help prevent pathogen introduction, while internal biosecurity measures are those that might reduce pathogen transmission within facilities. Two principal clusters of internal and external biosecurity measures were generated respectively for attitudes and practices (Metaxa, 2008). In addition, for attitudes questions, a third category was about general management.

To evaluate external biosecurity for farmers’ attitudes, we grouped attitudes towards stocking together with equipment and disinfection. Attitudes towards internal biosecurity included detection, prevention, and treatment of fish diseases. Early detection of disease included daily records on water quality and fish mortality, pond checks in the morning, measures taken before significant weather events (i.e. sudden rainfall and atmosphere temperature change), and experience in pond management. We used 5 questions to determine attitudes toward disease prevention: decreasing fish density, improving water quality, improving harvest procedures, removal of dead fish, and periodic removal of grass around ponds. Attitudes towards treatment included uses of prophylactic antibiotics, probiotics, and Chinese herbs. Seven questions were grouped into attitudes towards general management: pond disinfection, fish health status, disinfection of feed machinery, water quality improvement, oral medications, early diagnosis of diseases, and biosecurity measures.

Practices questions included external and internal biosecurity. External biosecurity practices included 3 components: stocking, traffic control, and equipment sharing and disinfection. Stocking practices included questions about information collection on fingerling suppliers, quarantine and disinfection of new fingerlings, separation of fish between different year classes, and whether stocking fish from farms with disease-outbreak history. Traffic control consisted of questions about whether farmers helping neighboring outbreak farms, disinfecting roads shared with diseased farms, and visiting other farms. For equipment sharing and disinfection, we asked 3 questions about whether farmers shared harvest equipment with other facilities, avoided sharing equipment with facilities where disease outbreaks were occurring, and disinfected harvesting equipment.

Most questions about internal biosecurity practices were paired with attitudes questions about internal biosecurity, including 3 subcategories of practices: detection, prevention, and treatment of fish diseases.

3.3.4.3. KAP index

In order to assess farmers' KAP based on our grouping of KAP questions, we developed an index from answers to each question by assigning a score and then compute percentage- scores for each sub-category, category, and overall biosecurity of three sections of KAP.

All knowledge questions were weighted by dichotomized scales scores of 0-1, and all attitudes questions were weighted using 0-1-3-5 scores. In the practices section, responses to external biosecurity questions were equally weighted by trichotomized scores of 0-1-2. For scaling of internal biosecurity questions about practices, dichotomized scores were used, except for 3 questions with multiple answers.

Two questions were excluded from scaling because of their redundancy or irrelevance to the pond aquaculture done by the farmers interviewed, which were “Keep different year class of fish in one pond” and “Periodically clean the feed machine”.

3.3.4.4. Data analysis

Descriptive analyses of surveyed data were performed using STATA 13(Stata Corp., College Station, TX, USA), and included demographics of respondents, responses rates, and quantitative measures of biosecurity levels of yellow catfish farmers based on scores from the 3 sections of KAP survey.

I used Fishers' exact tests to compare homogeneity of original responses, and to check whether answers to each question were consistent among farmers. To minimize information bias caused by missing data, a scaling process was done, considering best-case and worst-case scenarios. Computation of worst-case scenarios was done based on the following 4 equations (Equations 1-4).

Maximum score of subcategory = sum of all highest scores of each question a farmer could get   Eq.1

Subcategory score = Sum of original scores of each question / maximum score of subcategory   Eq.2

Category score = Sum of each subcategory score under this category/ maximum score of category   Eq.3

Overall final score = Sum of each category score / maximum score of all categories   Eq.4

Computation of scores was done and checked in Excel and Minitab, Release 16.0 (Minitab Inc., State college, PA, USA). The classification of farmers' KAP was defined using score boundaries of "Good" (≥ 75%), "Fair" (50% to75%), and "Poor" (< 50%) (Marian and Osuu Joy, 2012; Yimer, 2014). In the best-case scenario, non-response questions were not included in the denominator for calculation of the maximum scores of each subcategory.

Statistical analysis was conducted separately for each province. The median and percentage of scores were calculated for each subcategory, category, and overall, for KAP. To compare farmers' KAP scores from two provinces, Mann-Whitney U tests were used for the comparisons of medians, and chi-square tests for percentages of score classification.

I also computed weighted kappa using trial version of MedCalc 14.12.0 software (MedCalc Software, Acacialaan, Ostend, Belgium), to evaluate the agreement of each farmer's responses to practices questions for equipment sharing and visit control for internal validation.

3.4. Results

I interviewed 87 farmers in Guangdong (44 farms) and Zhejiang (43 farms) during April and May 2014 (Table 3.1). Of the 87, 37 (42.5%) farmers did not complete the KAP survey and, therefore, were excluded from data analysis.

3.4.1. Demographic information

Demographic information for the 50 respondents is summarized in Table 3.2.

Gender and age. There was a single female respondent in Guangdong, and no female respondents in Zhejiang. Due to the small sample sizes, two age-group categories were created: 25-50 years, and 51-65 years. The mean age of farmers in each province was 52 years, with ranges of 30-64 in Guangdong and 25-61 in Zhejiang.

Education. Most yellow catfish farmers in both provinces had elementary-level education. In Guangdong, 12% of farmers had not completed elementary school. A slightly higher percentage of middle school and high school education was found in Zhejiang. One farmer in Zhejiang had a college education.

Aquaculture experience was defined as the number of years involved in aquaculture production (not limited to yellow catfish culture). In this study, farmers in Guangdong and Zhejiang averaged 15 years' experience in fish farming. Interestingly, almost 50% of respondents in

Guangdong only had 5-10 years' aquaculture experience, and might not have had any experience with freshwater aquaculture before they started growing yellow catfish (according to anecdotal notes). Overall, 22 percent of farmers reported having 21-35 years of aquaculture experience.

Training in aquaculture. A slight, but not statistically significant, difference was shown between percentages of farmers with formal aquaculture training in Guangdong (10%) and Zhejiang (33%).

3.4.2. Descriptive analysis of farmers' original responses

Farmers' response rates and percentages of response options are reported in Tables 3.3-5.

3.4.2.1. Knowledge

Causes of fish diseases. Twenty percent of farmers in Guangdong and 10% in Zhejiang had poor knowledge of causes of aquatic infectious diseases (Table 3.3). Knowledge of viral diseases of fish was totally new to 30% of farmers in Zhejiang. Most farmers, in both provinces, were familiar with fish diseases related to suboptimal water quality, and infections of bacteria, parasites, and fungi (Table 3.3).

Causes of disease introduction and spread. Farmers had limited knowledge about roles of the following transmission pathways of fish diseases (Table 3.3): equipment being shared, vehicles control, biological vectors, disinfection at farm entrance, and stocking fish with mixed-year classes. More respondents in Guangdong had an awareness of whether or not 2 practices (p=0.019) - turtle farmers picking up dead fish from different farms, and mixing year classes during stocking - could cause introduction and spread of fish diseases (Table 3.3).

3.4.2.2. Attitudes

(I) Attitudes towards general practices of disease control and pond aquaculture

In Guangdong, 61% of farmers responded with “not sure” for the importance of general concept of farm biosecurity, while 81% said the same in Zhejiang. More positive attitudes toward early diagnosis were evident in Zhejiang than in Guangdong. In both provinces, 3 strategies of fish health management- water quality improvement, medication through feed, and pond disinfection- were identified as most useful by the majority of farmers. Most farmers, in both provinces, thought the role of feed management was useful in disease control, including feed quality, feeding frequency, and feed usage. In contrast, 10% of farmers responded that feed management was not useful for disease control (Table 3.4).

(II) Usefulness of specific routine management practices

Stocking strategies. Almost 50% of farmers from both provinces did not think that it was useful to have fingerling quality control by government agencies. Similar percentages of farmers regarded it as useful to know the supplier farms of fingerlings, and to quarantine and disinfect fingerlings before stocking.

Farm records. In Guangdong, over 70% of farmers thought it was useful to have daily records of either fish mortality or water quality; in contrast, only 50% of farmers in Zhejiang had a similar attitude toward record-keeping (Table 3.4).

Stress minimization. There was a wide range of opinions about the usefulness of separation between different year classes for disease control. Most farmers thought reducing density was useful for fish health (Table 3.4).

Disinfection. Most farmers responded it was moderately useful to disinfect roads if they were shared with an outbreak farm. In contrast, most farmers did not think it was useful to avoid sharing equipment with farms keeping infected fish. In addition, most farmers did not think it was useful to disinfect harvest nets with chemicals, but did use sunlight to dry nets when possible.

Treatment of diseases. Over 30% of farmers surveyed did not regard it useful to apply antibiotic treatments to decrease mortality at the start of a disease outbreak. The use of probiotics and Chinese traditional medicines was seen as acceptable by fish farmers.

Other routine practices related to fish disease prevention. Most farmers (Table 3.4) responded that the fish health management practices of water quality improvement, timely response to weather changes, and pond checking in the morning, were "moderately useful" or "very useful." More than 80% of farmers did not think that harvest procedures needed modifications to prevent disease.

3.4.2.3. Practices

(I) Practices to prevent introduction of disease agents

Disinfection. Compared with 35% in Guangdong province, 63% of farmers in Zhejiang stated that they helped their neighbours during disease outbreaks. Almost all farmers shared harvest equipment with other farms, while most of them (87% in Zhejiang and 90% in Guangdong) never disinfected harvest equipment between uses (Table 3.5).

Stocking strategies. More than two-thirds of farmers in both provinces did not use isolation and quarantine for new fingerlings, but most farmers in Guangdong (85%) and Zhejiang (87%) disinfected newly-introduced fish (Table 3.5). According to anecdotal information, some farmers disinfected new fingerlings using immersion in sodium chloride solutions.

(II) Farm records

More than 50% of farmers in both provinces did not maintain daily records on either water quality or fish mortality (Table 3.5).

(III) Routine practices for disease management on farms

Stress minimization. Most farmers (79% in Guangdong and 68% in Zhejiang) stocked the single year-class of yellow catfish in the same pond and did not fallow between year-classes. Several pond management practices related to stress minimization were used by many farmers, including improvement of water quality, response to weather changes, and pond checking in the morning. Timely removal of dead fish was also done by most farmers (84% in Guangdong and 97% in Zhejiang) when excessive mortalities occurred in a pond. More farmers made efforts to improve harvest procedures in Zhejiang (41%) than in Guangdong (5%) (Table 3.5).

Treatment of fish diseases. Antibiotics were used to prevent disease occurrence and reduce losses from fish disease outbreaks. Two other types of chemical treatments were often used in both provinces: probiotics (90% of farmers) and Chinese traditional herbs (70% of farmers).

Response actions for disease outbreaks on own facilities. Only 37% farmers in Guangdong and 19% in Zhejiang reduced fish stocking density after serious disease outbreaks in the previous production cycle. Most farmers avoided sharing equipment with other facilities if they knew the latter potentially had infected fish (Table 3.5).

Almost no farmers disinfected roads shared with disease outbreak farms. Similar percentages of farmers in both provinces often visited neighboring farms, even after knowing their disease status. Only a few farmers prohibited visits of neighbors from outbreaks farms (Table 3.5).

Other routine fish health management practices. There was no live fish movement between ponds on different farms in each province. About 32% more farmers in Zhejiang stated a tendency to rely on their own aquaculture experience to make decision of fish health management than did farmers in Guangdong (Table 3.5). Routine removal of grass growing around ponds was practiced "often" by two-thirds of Zhejiang farmers, while in Guangdong over half of farmers "seldom" did so (Table 3.5).

3.4.3. Evaluation of the biosecurity of yellow catfish farmers after scaling

KAP scores were reported as median and percentage for the "good-fair-poor" response categorization by subcategory, category, and overall levels for each attribute, with worst-case scenario (Tables 3.6-8). For knowledge scores without missing data, there is no need to consider both best- and worst-case scenarios.

3.4.3.1. Knowledge scores

Most farmers had a fair level of knowledge about external and internal biosecurity (Table 3.6). Only 12% of respondents had a poor level of knowledge. Knowledge of transmission routes for fish diseases was poor for approximately 50% of farmers in both provinces (Guangdong 47%, Zhejiang 58%). In spite of the poor understanding of causes of disease spread, farmers in both provinces had good levels of knowledge (Guangdong 89% and Zhejiang 87%) of primary causative agents of fish diseases.

3.4.3.2. Attitude scores

The median score for attitudes, for all farmers in the study, was 58 out of 100, according to our worst-case scenario calculation. A fair level of attitude was achieved by 64% of respondents.

Attitudes towards external biosecurity. Farmers had poor attitudes towards external biosecurity measures. The overall median attitude score for external biosecurity was 49, calculated from the worst-case scenario, which can be further explained by farmers' poor scores for their attitude towards stocking, and sharing and disinfection of equipment. However, farmers in Guangdong had a slightly higher median score (53) for attitudes toward stocking than those in Zhejiang (47) (Table 3.4).

Attitudes towards internal biosecurity. In the worst-case scenario, there was no significant difference between overall median scores of farmers in Guangdong and Zhejiang, and the overall percentage of farmers that scored fair was 64%. Consistent with this finding, the majority of farmers had positive attitudes to early disease detection. Farmers reported slightly negative attitudes towards treatment of fish diseases, and 50% of farmers in the study had a poor score when asked about usefulness of disease treatment methods (Table 3.7). In the worst-case scenario, farmers in Zhejiang had a higher median score (60) for their attitude towards fish disease treatments than those in Guangdong (40) (Table 3.7).

Attitudes towards general management practices. Overall medians for farmers' attitudes towards general management practices were different in best- and worst-case scenarios: 59 and 33, respectively. Sixty percent of farmers were classified at a fair level for their attitude towards general management practices in the study (Table 3.7).

3.4.3.3. Practices scores

All farmers were classified as poor or fair for their compliance with over-all farm-level biosecurity measures. In the worst-case scenario for all respondents, overall median score for practices was 49 out 100. A poor level of practices was evident in 52% of respondents, and the median score of poor levels was only 42.

External biosecurity practices. About 70% of farmers scored less than 50 in utilization of external biosecurity barriers (Table 3.8). Most respondents (78%) were evaluated at a poor level for this category. Farmers in both provinces were categorized as good for their practices in fish stocking. Sixty-eight percent and 81% of farmers from Guangdong and Zhejiang, respectively, had poor levels of traffic control (Table 3.8). The median score for traffic control when computed in the worst-case scenario, was 25. Farmers in both provinces had extremely low scores for equipment sharing (Table 3.8). The overall median score was 20, in both scenarios, for farmers' practices in disinfection of equipment, of which 92% were evaluated as poor.

Internal biosecurity practices. Farmers in Zhejiang had a significantly higher overall median score in this category than those in Guangdong, when calculated in the best-case scenario ($p=0.01$), but not in the worst-case scenario (Table 3.8). Median values were similar when separate calculations were done for the 3 subcategories in both scenarios. Distributions of score percentages were significantly different between farmers in the two provinces when calculated in the worst-case scenario. Inconsistency of percentages in the 3 different levels was observed for the same subcategories, using both scenarios. Farmers' had a median score of 57 for their early detection of fish diseases. Results for how farmers prevented disease spread within facilities were very similar to those of attitudes towards disease prevention. According to the worst-case scenario, farmers achieved a median of 56 for pond management practices. The median score of disease treatment practices in the two provinces was 67, including medication with antibiotics, probiotics, and Chinese traditional medicine. Fifty percent of farmers scored fair in this subcategory, indicating that medication was one of the priority strategies for disease mitigation.

3.4.4. Consistency of farmers' responses to repeated questions

When farmers were asked: "Do you help neighboring farms when they need help for a disease outbreak?" and "Do you go to neighboring farms with disease outbreaks (occurring or just having occurred)?" weighted kappa of inter-rater agreement of responses from these two questions was 0.014 (95% confidence interval, -0.170 to 0.198), indicating poor agreement between similar questions. Some farmers might have only visited to ask for information about disease outbreaks and not been involved in removal of dead fish, however, this was unlikely to explain the marked inconsistencies in answers. The second pair of questions included: "Do you share harvest equipment with other farms?" and "Do you avoid sharing equipment with farms of disease outbreak of yellow catfish?" I reversed the original responses, and frequencies were reported as how often farmers shared equipment with outbreak farms. Weighted *kappa* for the second group comparison was -0.155 (95% confidence interval, -0.031, 0.027), which also indicated inconsistencies in answers. Farmers did not regard it as risky to share, or could not afford to own, particularly expensive equipment and had to share with other farms, even though some respondents acknowledged that it might be risky for the introduction of pathogens.

3.5. Discussion

To my knowledge, this is the first study of biosecurity in small-scale, freshwater pond aquaculture of fish in China. Results of this survey indicate that biosecurity practices for disease control could be improved on many yellow catfish farms in the 2 provinces studied.

Aquaculture has been part of the social, economic, and cultural fabric of the agricultural society in China for centuries (Halwart and Overton, 2001). However, freshwater fish aquaculture has been intensified in recent years, and there have been anecdotal reports from farmers that mortalities in farmed fish have increased. Although it is not known that whether this can be attributed to infectious diseases or other factors, intensification of animal production systems often increases likelihood of pathogen transmission for host-density dependent diseases (Stendera et al., 2012). Compared with traditional extensive or semi-intensive aquaculture systems, there is an increased risk of disease occurrence because of increased stress level in the intensive aquaculture system, especially given a lack of biosecurity measures. In order to address biosecurity implementation on freshwater pond aquaculture farms in China, specific deficiencies were identified in knowledge, attitudes and practices of farmers.

Farmers' basic knowledge of pathogens was good; however, they had limited understanding of pathogen transmission. Interestingly, knowledge scores suggested that education on biosecurity could be improved. The larger issue, though, may be farmers' understanding of the benefits of biosecurity and its implementation. As fish farming in China is typically a life-long occupation, farmers have accumulated decades of experience in traditional extensive aquaculture settings. However, in this study, the majority of farmers lacked knowledge about modes of introduction and spread of aquatic disease microbes in modern intensive aquaculture. Educational campaigns should, therefore, target these topics.

Low-level compliance with biosecurity practices was found for aquatic animal health management on these yellow catfish farms in the study, which might be explained by farmers' incomplete understanding of the relationships between pathogens, animal hosts, and environment (Yanong and Erlacher-Reid, 2012).

Overall scores for attitudes towards biosecurity were slightly higher than practices scores, although they were still low. Answers to practices questions reflected the need to improve aquatic animal health management practices, and were in line with 4 major risk factors for disease introduction and spread in aquaculture facilities: fish movement, general fish health issues, equipment/vehicles sharing, and vector control (Dvorak, 2009). Questions about attitudes and practices being divided into external and internal measures will be useful to better understand where improvements could be made.

For external biosecurity, most farmers had poor scores for both attitudes and practices. Low-risk stocking and disinfection of harvest equipment were thought to be important by farmers, but few, particularly those with little formal education, chose to adopt them. Most respondents indicated that it was useful to be aware of the health status of hatcheries and fingerlings before stocking; however, this was not enforced and, given realistic limitations and lack of a policy framework, most farmers did not think it was practical for government or a third party to regulate fingerling quality. Other practices were regarded as potentially high risk but were still used by most farmers;

these included frequent visits to other farms, helping other farmers with disease outbreaks, and sharing harvest equipment. There were likely cultural, social, and financial reasons for farmers' practices with potential risks of introducing pathogens into their farms. Because of the labor-intensive nature of aquaculture practices, farmers also had to help each other with measures taken during mass mortalities, i.e. removal of dead fish from ponds.

Inconsistencies between attitudes and practices in aquatic animal biosecurity measures have also been reported in prior research done on freshwater recirculation aquaculture facilities in North America (Delabbio et al., 2003). This may reflect a need for improved knowledge on the benefits of biosecurity and or it may be an issue with implementation costs of such practices.

For internal biosecurity, nearly 50% of farmers regarded proactive detection of disease to be important in disease control, but only a few indicated that they had a system in place for early detection of disease. Routine record-keeping was suggested by some aquatic feed companies, but most farmers neglected both record taking and data sharing. Overall, very few farmers believed the suggested measures of disease prevention were very important. Despite that, most farmers practiced only several common disease management strategies, such as improving water quality, removal of dead fish during high mortality events, and cleaning grass around ponds.

Very few farmers had good scores for their attitudes towards treatments with antibiotics, probiotics, and traditional Chinese medicine, which suggested that few regarded treatments as very effective against disease. However, most farmers also indicated that they used those 3 types of chemical treatment products. This discrepancy in attitudes and practices scores may be because treatments are sometimes applied without knowing the cause of mortality, which could result in the impression that treatments have an overall poor response. For example, due to limited on-site laboratory diagnoses of fish disease, many farmers might treat parasitic problems with antibiotics, which were only effective against bacterial diseases, thus giving the impression that antibiotics were not effective.

This study had some limitations. Only 2 provinces with yellow catfish aquaculture were selected in China because of budgetary and logistical constraints, and most farmers surveyed had small-scale production systems. Based on information provided by Chinese aquaculture experts, we assumed that surveyed farms were representative of yellow catfish production in these 2 provinces. Consequently, the study results likely might not apply to large, corporate yellow catfish farms in these provinces that operate under different economic realities. Limited sample size was another important constraint of this study, and this was exacerbated by missing data. Further, it was not possible to obtain a list of farmers and use probability sampling. Convenience sampling was used to increase response rates because local farmers were not used to talk to an interviewer without an introduction from someone they knew. In addition, since it was an unexpected rainy season when I conducted the survey, farmers had limited time to answer questions due to the need to closely monitor fish health status during those rainfall events.

The first author conducted interviews with linguistic assistance from local people, especially in Guangdong province, as it was difficult to build sufficient rapport using Mandarin as an alternative to local dialects. Farmers tended to respond faster and were more willing to continue the interview if they were able to communicate in their local dialects. Given the context of interviewer and interviewees, courtesy bias might have occurred in the study (Launiala, 2009), and might explain why farmers gave contradictory answers when they were asked about their attitudes and actual practices with regard to the same biosecurity measures.

The focus of biosecurity is on exclusion of unwanted pathogens (Lightner, 2003), and practices that reduce the risk of introduction of disease microbes are needed in intensive aquaculture facilities. Farmers' attitudes and practices scores for external biosecurity were generally poorer than those for internal biosecurity. This might be related to farmers' less ability or unwillingness to control external biosecurity risk factors. Even though there are many logistical factors limiting compliance with external measures, farmers should try to improve them to prevent catastrophic losses. If external measures are difficult to implement, reducing pathogen spread within farms should be considered in an introduction event. The latter was identified as need improvement in the study.

China has been the global leader in freshwater fish production in recent decades, however, there have not been concerted efforts to implement science-based biosecurity practices for freshwater finfish pond aquaculture (Perera et al., 2008). In comparison with basic biosecurity principles used in finfish species in other countries, the overall level of biosecurity practices in the provinces surveyed was relatively low, and highlighted a possible limiting factor for sustainable growth of intensive freshwater aquaculture in China.

Biosecurity was a new concept for most farmers in our study, and private fish clinic managers whom I communicated with also confirmed that the term "biosecurity" was new to them. Farmers might not have applied on-farm biosecurity measures because they did not realize that their traditional aquaculture experience might benefit from biosecurity practices, especially as farming efforts begin to intensify. This may explain the low compliance and different attitudes towards these practices. There is still a gap between conceptual realization of disease management and increased production, and improved welfare for many farmed freshwater species in China. In order for farmers to implement biosecurity measures, they need to know the basic principles of biosecurity and be convinced (about attitude) that they will reduce risk of disease, and then they need the ability to implement the practices. Most biosecurity questions in this study were based on practices adopted in modern aquaculture facilities rearing salmonid species. These biosecurity principles have been shown to apply to intensive farming in many commercial aquaculture systems, but specific practices may not be completely applicable to pond aquaculture.

This KAP survey was a useful tool to better evaluate the knowledge, attitudes, and practices of biosecurity measures on small-scale fish farms in two provinces in China. Based on our assessment, there appears to be a need for educational programs, especially regarding pathogen transmission; however, perhaps even more importantly, understanding why farmers did not implement certain practices despite knowing their importance, may help to identify critical areas for education and training. Based on the findings of our economic study (Jia et al. 2015 submitted), the following factors-the types of aquaculture facilities, and the economic efficiency of these facilities- may play a role in the poor implementation of biosecurity found in this study.

A socio-economic analysis of different biosecurity practices would have been useful for understanding why farmers did or did not adopt certain strategies, but this was not feasible because of the limited number of farms with matched biosecurity and returns and costs data. Although I did not adopt the social-economic framework (Keizer, 2005) in this study, one advantage of a KAP study was that it allowed formulation of hypotheses and provided important directions for future studies. Even with the limited number of freshwater finfish farmers in our study, I found there was limited knowledge transfer about prevention of aquatic infectious diseases.

3.6. Conclusions

Using a KAP survey of farm-level biosecurity, I quantified behaviors and perceptions of fish farmers culturing yellow catfish in 2 provinces in China. Farmers' practices were, overall, not in complete compliance with biosecurity principles, especially in regards to prevention of pathogen introduction and spread. It is recommended by this study that current farm-level biosecurity practices in finfish aquaculture in China be improved through investment in education and training, in collaboration with academic and industry partners. The inconsistencies between practices and attitudes may indicate that it would be feasible to improve farmers' implementation of biosecurity measures at the farm level. Future research is necessary to define specific biosecurity measures applicable to intensive pond aquaculture.

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Table 3.1 Source population and sample size of yellow catfish farms investigated in Guangdong and Zhejiang provinces in 2014.

Province	**Prefecture**	**County**	**Estimated no. of farms in county**	**Total number of farms in sample frame**	**Total no. of farmers interviewed**	**Total no. of farms analyzed**
Guangdong	Foshan	Nanhai	3000	50	44	19*
Zhejiang	Huzhou	Deqing	800	15	10	8
		Nanxun	1000	20	19	13
		Wuxing	1000	20	14	10

Note: * Data for a single farmer in Guangdong Province was excluded from KAP scaling process due to his low response rates.

Table 3.2 Demographic information of yellow catfish farmers investigated in Guangdong and Zhejiang provinces in 2014.

	Guangdong (n=19)		**Zhejiang** (n=31)	
	Response rate (%)	N (%)	Response rate (%)	N (%)
Gender	100		100	
Female		1 (5.3)		0
Male		18 (94.7)		31(100)
Age (years)	100		100	
I [25, 50]		7 (36.8)		13 (41.9)
II (>50, 65]		12 (63.2)		18 (58.1)
Education	100		97	
I (< elementary)		3 (15.8)		0
II (elementary school)		10 (52.6)		15 (50.0)
III (middle school)		4 (21.1)		12 (40.0)
IV (>=high school)		2 (10.5)		3 (10.0)
Experience (years)	100		97	
I [5, 10]		9 (47.4)		12 (40)
II (11-20]		5 (26.3)		12 (40)
III (21-35]		5 (26.3)		6 (20)
Training	100		97	
With training		2 (10.5)		10 (33.3)
Without training		17 (89.5)		20 (66.7)

Table 3.3 Farmers' knowledge of aquatic infectious disease control on yellow catfish farms in Guangdong and Zhejiang provinces in 2014.

Questions about farmers' knowledge	Guangdong			Zhejiang		
	Yes	**No**	**Not sure**	**Yes**	**No**	**Not sure**
Have you heard of the following causes of diseases in fish?						
Bacterial diseases	0.95		0.05	0.87	0.13	
Viral diseases	0.85	0.15		0.71	0.29	
Fungal diseases	0.95	0.05		0.9	0.1	
Parasitic diseases	0.95	0.05		0.94	0.06	
Poor water quality	1			0.87	0.13	
Aquatic infectious diseases	0.8	0.15	0.05	0.9	0.1	
Will the following practices cause introduction and spread of disease?						
Sharing equipment	0.5	0.3	0.2	0.48	0.45	0.07
Use of same feed company truck with farms of disease outbreak	0.1	0.8	0.1	0.13	0.87	
Shared same road with farms of disease outbreak	0.3	0.45	0.25	0.23	0.74	0.03
Wild birds picking up dead fish from pond	0.45	0.25	0.3	0.58	0.29	0.13
Visited a fish vet clinic and returned without disinfection	0.4	0.35	0.25	0.42	0.52	0.06
Water seepage through ground between ponds	0.9	0.05	0.05	0.77	0.19	0.03
Turtle farmers collecting dead fish**(p=0.02)	0.7	0.05	0.25	0.39	0.39	0.22
Mixing of different year classes of fish**[a](p=0.001)	0.3	0.2	0.5	0.06	0.71	0.23

Note: [a,]** (p<0.05), represented heterogeneity of percentages of farmers responses in Zhejiang and Guangdong.

Table 3.5 Farmers' attitudes towards disease control measures on yellow catfish farms in Guangdong and Zhejiang provinces in 2014.

	Guangdong					Zhejiang				
Items	**Response rate (%)**	**VU[a]**	**MU**	**NU**	**NS**	**Response rate (%)**	**VU**	**MU**	**NU**	**NS**
Importance of following general practices										
Pond disinfection	90	0.78	0.22			100	0.55	0.39	0.06	
Feeding management (time and choice of feed)	90	0.5	0.44	0.06		100	0.29	0.61	0.1	
Disinfection of feeding equipment [b]** (p= 0.043)	90	0.11	0.06	0.83		100	0.1	0.35	0.55	
Water quality improvement	90	0.94		0.06		100	0.84	0.16		
Medication through feed [b]** (p= 0.022)	90	0.67	0.22	0.06	0.06	100	0.26	0.48	0.23	0.03
Early diagnosis [b]*(p=0.054)	90	0.39	0.22	0.17	0.22	100	0.55	0.32	0.13	
Biosecurity measures	90	0.22	0.06	0.11	0.61	100	0.06	0.1	0.03	0.81
Usefulness of following routine practices										
Be aware of where the source of fingerlings is located	100	0.79	0.11	0.05	0.05	94	0.62	0.31	0.03	0.03
Quarantine & disinfection for fingerlings before stocking	100	0.79	0.11	0.05	0.05	94	0.62	0.31	0.03	0.03
Government controls quality of fry or fingerlings	100	0.21	0.21	0.42	0.16	94	0.03	0.17	0.59	0.21
Keep daily water quality records for early detection	90	0.44	0.33	0.22		94	0.48	0.17	0.34	
Keep daily mortality records for early detection	100	0.58	0.21	0.16	0.05	94	0.28	0.28	0.21	0.24
Keep same year class of fish in one pond	80		0.33	0.33	0.33	90	0.14	0.29	0.43	0.14
Fallow between year classes	80	0.27	0.33	0.4		90	0.21	0.43	0.36	
Decrease fish density	80	0.53	0.13	0.21	0.13	94	0.28	0.28	0.34	0.1

Remove dead fish from pond when big mortality [b]*	80	0.86	0.07		0.07	87	0.59	0.3	0.11	
Disinfect road shared with outbreak farm	80	0.2	0.73	0.07		97	0.04	0.83	0.13	
Disinfect harvest net before harvesting fish [b]*	90			0.83	0.17	94	0.1	0.24	0.55	0.1
Don’t share equipment with facilities of disease outbreak	80	0.27	0.13	0.47	0.13	97	0.1	0.2	0.57	0.13
Use antibiotics ahead of mortality	80	0.19	0.44	0.25	0.12	97	0.4	0.27	0.3	0.03
Use probiotics for water quality improvement	80	0.19	0.44	0.13	0.25	97	0.27	0.5	0.17	0.06
Use Chinese herbs	80	0.19	0.44	0.13	0.25	97	0.2	0.4	0.27	0.13
Water quality improvement	80	0.81	0.19			94	0.86	0.03	0.03	0.07
Learn to improve harvesting procedure [b]*	80		0.13	0.67	0.2	84	0.31	0.15	0.46	0.08
Be able to respond to weather changes over time	80	0.73	0.13	0.07	0.07	84	0.92	0.04	0.04	
Check ponds early in the morning	80	1				84	0.84	0.08	0.08	
Apply extensive experience in pond management	80	0.8		0.13	0.07	84	0.73	0.04	0.12	0.12
Periodically remove grass near ponds	80	0.33	0.27	0.13	0.27	84	0.19	0.31	0.42	0.08
Periodically clean feed machine	80			0.2	0.8	84	0.04	0.04	0.19	0.73

Note: [a].VU= very useful; MU= moderately useful; NU= not useful; NS= not sure.

[b]. “*” (0.1>p>0.05) and “**” (p<0.05), represented heterogeneity of percentages of farmers responses in Zhejiang and Guangdong.

Table 3.6 Farmers' practices of disease control on yellow catfish farms in Guangdong and Zhejiang provinces in 2014.

Items	Guangdong				Zhejiang			
	Response rate (%)	**O/Y[a]**	**S/NA**	**Nev/No**	**Response rate (%)**	**O/Y**	**S/NA**	**Nev/No**
Prevention of introduction of disease agents								
Do you help other farms during their high fish-mortality events?	100	0.35	0.35	0.3	97	0.63	0.17	0.2
Do you disinfect harvest equipment?	100		0.1	0.9	97	0.1	0.03	0.87
Do you share harvest equipment with other facilities?	100	0.9	0.05	0.05	97	0.77		0.23
Do you collect information about the hatchery you buy from?	100	0.55		0.45	97	0.5	0.23	0.27
Do you disinfect new fingerlings?	100	0.85		0.15	97	0.87	0.03	0.1
Do you isolate and monitor new fish?	100	0.3	0.1	0.6	97	0.33	0.07	0.6
Farm records								
Do you keep detailed daily records on water quality?	100	0.25	0.1	0.65	97	0.37	0.1	0.53
Do you keep detailed daily records on fish mortality?	100	0.35	0.1	0.55	97	0.1	0.13	0.77
Routine practice for disease management on farm								
Do you keep the same year class of fish in one pond?	95	0.79	0.21		100	0.68	0.32	
Do you fallow between year classes?	95	0.05	0.95		100	0.16	0.77	0.06
Do you decrease fish density if increased mortality in previous year?	95	0.37	0.63		100	0.19	0.81	
Do you disinfect the road shared with the outbreak farm?	95	0.05	0.84	0.11	100	0.03	0.97	
Do you avoid sharing equipment with facilities with infected fish?	100	0.75	0.1	0.15	97	0.9	0.07	0.03
Do you remove dead fish from pond when mortality is above normal?	95	0.84	0.16		94	0.97		0.03

Do you use antibiotics prophylactically as a disease prevention measure?	95	0.63	0.26	0.11	100	0.71	0.29	
Do you use probiotics for water quality improvement?	95	0.89	0.11		100	0.9	0.1	
Do you use Chinese herbs?	95	0.68	0.26	0.05	100	0.71	0.26	0.03
Do you pay attention to improve water quality?	95	0.95	0.05		100	1		
Do you learn to improve harvesting procedure? [b]**(p= 0.008)	95	0.05	0.89	0.05	94	0.41	0.55	0.03
Do you pay attention to weather changes in time?	95	0.79	0.16	0.05	94	0.97	0.03	
Do you check pond early in the morning? [b]** (p=0.020)	95	0.79	0.21		94	1		
Do you have extensive experience in pond management?	95	0.68	0.32		94	0.9	0.1	
Do you periodically clean the grass near ponds?	95		0.58	0.42	94	0.66	0.34	
Do you periodically clean the feed machine?	95		0.95	0.05	94		0.9	0.1
Have you visited other farms during past 2 weeks?	100	0.75	0.1	0.15	97	0.9	0.07	0.03
When mortality occurs on neighboring farms								
Do you visit mortality farms after knowing their mortality?	90	0.72	0.28		94	0.76	0.24	
Do you forbid farmers from mortality farms to visit your farm?	90		1		94	0.03	0.97	
Do you move fish from mortality farms to your farm?	90		1		94		1	

Note: [a]. O= often; S= seldom; Nev= never; Y= yes, NA=not always.

[b]. "**" ($p<0.05$), represented the heterogeneity of percentages of farmers responses in Zhejiang and Guangdong.

Table 3.7 Knowledge scores for biosecurity of yellow catfish farmers in Guangdong and Zhejiang provinces in 2014.

		Guangdong				Zhejiang			
Categories		**Good**	**Fair**	**Poor**	**Overall**	**Good**	**Fair**	**Poor**	**Overall**
Basic knowledge	Median	100	67	33	100	100	58	8	100
	Percentage (%)	89	5	5		87	6	6	
Causes of disease spread	Median	75	63	25	50	88	50	25	38
	Percentage (%)	21	32	47		3	39	58	
Overall	Median	86	64	32	64	93	64	32	64
	Percentage (%)	32	58	11		3	84	13	

Table 3.8 Attitude scores for external/internal biosecurity measures and general management of yellow catfish farmers investigated in Guangdong and Zhejiang provinces in 2014 (worst-case scenario).

			Guangdong				**Zhejiang**			
Categories	**Subcategories**		Good	Fair	Poor	Overall	Good	Fair	Poor	Overall
External biosecurity	Stocking	Median [b**]	75	60	35	60	90	58	30	45
		Percentage (%)	16	58	26		10	39	51	
	Equipment &disinfection	Median	[a]	54	43	49	91	57	46	49
		Percentage (%)		42	58		4	35	61	
	Overall	Median		55	44	53	78	57	40	47
		Percentage (%)		63	56		3	39	58	
Internal biosecurity	Detection	Median	84	68	32	76	92	68	40	68
		Percentage (%)	53	26	21		39	42	16	
	Prevention	Median	80	64	0	60	78	60	22	60
		Percentage (%)	16	58	26		19	55	26	
	Treatment	Median	100	70	20	40	93	67	33	60
		Percentage (%)	5	32	63		13	42	42	
	Overall	Median	82	65	15	62	78	65	38	63
		Percentage (%)	21	53	26		19	55	26	
General management	Not applicable	Median	86	66	46	57	81	63	46	60
		Percentage (%)	16	58	26		6	61	32	
Overall	Not applicable	Median	75	64	34	61	85	59	39	58
		Percentage (%)	5	58	37		3	68	29	

Note: [a]. Blank: No observation in the corresponding category of the defined level;
[b].“**”denoted significant difference for the sub-categories between farmers in Zhejiang and Guangdong.

Table 3.4.4 Practices scores for external and internal biosecurity measures of yellow catfish farmers investigated in Guangdong and Zhejiang provinces in 2014 (worst-case scenario).

			Guangdong				**Zhejiang**			
Categories	**Subcategories**		Good	Fair	Poor	Overall	Good	Fair	Poor	Overall
External biosecurity	Stocking	Median	80	60	30	80	80	60	40	70
		Percentage (%)	58	21	21	[a]	42	45	13	
	Traffic control	Median	75	63	25	38		50	13	25
		Percentage (%)	5	26	68			19	81	
	Equipment & disinfection	Median			17	17		67	17	25
		Percentage (%)						10	90	
	Overall	Median		54	35	46		54	38	42
		Percentage (%)		26	74			19	81	
	Detection	Median	86	57	29	57	86	57	43	57
		Percentage (%)	26	47	26		13	65	23	
	Prevention	Median	83	61	44	67	83	56	39	56
		Percentage (%)	32	42	26		13	61	26	
Internal biosecurity	Treatment	Median	100	33	67	67	100	67	67	67
		Percentage (%)	47	37	16		45	45	10	
	Overall	Median	89	63	45	63	79	63	47	63
		Percentage (%) [b]**	21	58	21		3	87	10	
Overall	Not applicable	Median		60	42	51		54	42	49
		Percentage (%)		53	47			45	55	

Note: [a]. Blank: No observation in the corresponding category of the defined level;
[b]. "**"denoted significant difference for the sub-categories between farmers in Zhejiang and Guangdong

Chapter 4 Time-series regression analysis of logbook data: effect of predisposing factors and treatment on daily mortality count of pond-farmed grass carp (*Ctenopharyngodon idella*)

4.1. Abstract

Limited research has been done on the use of multivariable statistical methods to quantify the impact of risk factors on mortalities of farmed grass carp. The objective of the study was to investigate the association between pond-level daily mortality of farmed grass carp and predisposing environmental and husbandry factors denoted as air temperature fluctuation, movement of fish into and out of ponds, and 3 groups of treatments (antibiotics-antiparasitics), Traditional Chinese Medicine-probiotics, and water treatments). Based on the pond-level logbook data from a single farm in Guangdong province China in 2013, the two-stage time-series regression (TSR) analyses were carried out to estimate the lagged effect of the above predisposing factors on grass carp mortality. A negative-binomial distribution fitted with generalized linear model was used as the main model for within-pond estimation. Sensitivity analyses were conducted by substituting the 3 modeling components: distributional forms, number of spline knots, and autocorrelation term. The main model results indicated that the two risk factors could be responsible for the increase of mortality of grass carp: (1) movement-in of new fish during the previous 14 days; and (2) increasing temperature during the previous 7 days. Treatment with traditional Chinese medicine or probiotics was associated with a reduction of the incidence rate ratio of fish mortality. Sensitivity analyses suggested fairly good consistency of the estimation processes with different modeling components. Our findings highlighted the importance and usefulness of farm logbook data for the grass carp industry in China.

Key words: Time-series regression, grass carp, mortality, predisposing factors, treatment

4.2. Introduction

Grass carp (*Ctenopharyngodon idella*) is one of the most farmed warm-water species cultured in China due to its easy of domestication and acceptance in the marketplace (Cao et al., 2007; Yuan, 2007; FAO, 2014). Grass carp culture across the country is vulnerable to diseases, which have negative social, economic, food safety and environmental impacts. In order to maintain and improve on the success of grass carp culture in China, it is important to understand the causes of fish mortality in order to effectively develop effective mitigation and control programs (Wedemeyer, 1996).

Stress is now regarded as adaptive process of fish to survive with environmental stressors, rather than as only damage to fish health (Barton, 1997; Wendelaar Bonga, 1997; Chrousos, 1998). Ultimately, stress is directly or indirectly responsible for mortality in farmed finfish (Pickering, 1998; Braithwaite et al., 2014). Due to complexities of fish response to all kinds of stressors, predisposing stressors have been used to describe those factors triggering all the problems of aquatic environment and fish health, which finally disturb osmo-regulatory balance, and weaken the immune system, and thereby compromising disease resistance (Wendelaar Bonga, 1997; Soares, 2012; Narváez et al., 2015). Management practices and environmental factors can predispose fish to diseases and mortality, and predisposing factors have been described as those triggering the problems of aquatic environment and fish health (Blanco , Gibello, 2000; Soares, 2012; Narváez et al., 2015).

In this study, I targeted those predisposing factors-ambient temperature, handling (movement-in and movement-out)- that can predispose grass carp to mortality, and also treatment factors that may influence the mortality of grass carp. Ambient water temperature together with oxygen availability are the most influential environmental factors that affect aquatic organisms (Coutant and Talmage, 1976). These factors may have both acute and chronic effects, depending on the speed of temperature change and the ability for fish to acclimate to the change (Barton, 2002; Zhao et al., 2011; Chezik et al., 2014). Movement-in of new fish and movement-out of fish from grow-out ponds of grass carp often cause acute mortalities due to over-crowding, and similarly delayed mortalities due to introduction of pathogens, delayed physical injuries accompanied by concurrent environmental stressors (Barton, 2002).

In general, disease treatments are applied to control and reduce mortality. In some cases, however, treatments may not function due to improper diagnosis, resistance or improper dose usage and other limiting factors. Furthermore, chemical treatment may also have negative impacts, such as reduction of natural bacterial populations and algae (Pillay and Kutty, 2005). These impacts may lead to degradation of the pond's ecosystem, resulting in adverse health events (Moll, 1986).

Chinese carp farmers in rural areas rarely have timely and reliable access to diagnostic laboratories during mortality events. As a result, farm mortalities due to infectious disease can go undiagnosed. An alternative method of risk factor characterization is examination of production logbooks. These logs provide useful information for tracking fish health problems including mortality (Andersson and Olson, 1996; Walster et al., 2009). The primary objective of this study was to assess whether management practices (such as treatments and movements of fish) and environmental factors recorded during one production cycle at a single grass carp farm in the Guangdong province of China were associated with daily mortality counts. A secondary objective of the study was to explore the feasibility of time-series regression (TSR) methods for analysis of such data.

4.3. Materials and methods

4.3.1. Data source and data entry

Data used in the study were daily pond-level records from 14 ponds located on the same farm during a production cycle of grass carp in 2013. The farm was managed by a domestic aquatic feed company and used as a demonstration farm for clients to learn about best management practices in fish farming. The farm was comprised of 14 ponds of grass carp, 3 ponds of weather loach (*Misgurnus anguillicaudatus*) and other ponds for shrimp. I only included the grass carp ponds for this analysis. The14 ponds of grass carp included in the study were in the first year of production. There were also crucian carp *(Carassius auratus)*, silver carp (*Hypophthalmichthys molitrix*) and spotted silver carp (*Aristichthys nobilis*) in these ponds; however, we did not include mortality data from these species.

In this region of China, grass carp are usually cultured with other carp species in land-based pond farms in high-density systems. The systems are characterized by multiple fish movements, high feeding rates, and limited water exchanges. The daily records (logbooks) included information on: (1) movement-in of fish (weight and size of fingerlings or new fish of adult stage of multiple species); (2) movement-out of fish (species name, weight, and size); (3) treatment (chemical name and dose); and (4) water quality (temperature, pH, and ammonia etc.).

The original logbook data were recorded on paper by staff working for the aquatic feed industry during 2013, and data entry into Microsoft EXCEL 2010 (Microsoft, Redmond, WA, USA) was completed by Chinese university students with a formal education in aquaculture. This was done to reduce data entry errors and facilitate translation from Chinese into English of species' names, fish sources, treatments, and so on. The data-recording staff provided explanations when there were difficulties understanding the hand-written information in the logbooks, when needed. Quality control of data entry was supervised by feed company personnel.

4.3.2. Data preparation

4.3.2.1. Outcome variable

The outcome variable of the model in this study was daily mortality count of grass carp (*gcdeath*) on each day of each pond. Grass carp number (*gctotal*) on day 1, when movement-in was calculated, based on fingerlings' size and total weight. Except for Day1, the grass carp number on any given day was obtained by subtracting the daily mortality from the grass carp number of the previous day.

4.3.2.2. Predictor variables

Seven predictors were used to represent acute or delayed effects of the predisposing factors on fish mortality. Except for temperature, all movements and treatments of fish were coded as binary variables, which were assigned a value of 1 if fish were exposed to the corresponding practice within the respective time window, and otherwise a value of 0.

The 3 variables related to movement-in (movement-in) of new fish and movement out of fish, *mi3d, mi2w* and *mo3dm,* were defined as follows: (1) *mi3d*: whether there was movement of fish into the pond during the previous 3 days. I expected to see an increase in mortality within a short period of time after movement-in of fish if the mortality was associated with poor environment due to an increase in biomass. (2) *mi2w*: whether there was movement-in of fish during the previous 14 days. This is the time frame I anticipated would be required for an infectious pathogen introduction associated with a transfer of fish to have an effect. (3) *mo3dm*: whether there was movement-out of fish during the previous 3 days, except when the pond was within 10 days from the time of final harvest.

The 3 variables related to treatments, *atbp7d, ctpr7d* and *wimp3d*, were used to estimate the change in fish mortality post treatments: (1) *atbp7d*, whether either antibiotics or antiparasitics were used during the previous 7 days; (2) *ctpr7d*, whether either Chinese traditional medicine or probiotics were used during the previous 7 days; and, (3) *wimp3d*, whether water quality improvements were used during the previous 3 days.

The temperature effect was predicted by *tmax06***,** a continuous variable, indicating 7-day average of maximum daily atmospheric temperature. I consider this predictor is an important variable associated with mortality and might also be a potential confounding factor for other predictors. All historical records of atmospheric temperature for the study area were retrieved from online open-source climatic data available on the official website of Guangzhou City Meteorological Information Centre (http://www.gz121.gov.cn/gywm/sjkf/).

4.3.3. Explorative descriptive analysis

Production information was summarized for each pond, including dates of movement-in and movement-out, grass carp mortality, frequencies of movements and treatments of fish, and ambient temperature fluctuations.

Frequency distribution were used to explore the correlation between binary predictors, which was facilitated to understand the treatment practices of grass carp being treated using single methods of the 3 treatments or combined methods. The group means of atmosphere (*tmax06*) was also described for those observations with each of binary variable equal to 1 (*atbp7d, ctpr7d* and *wimp3d*) and those days with them equal to 0.

In order to compare median difference in pond-level mortality before-intervention and after-intervention of each movement and treatment of fish, I defined the following time windows for each management practice: (1) 3 days before and after movement-in of fish, movement-out of fish, and water improvement; (2) 7 days before and after treatment with antibiotics/antiparasitics and treatment with Chinese traditional medicine, and (3) 14 days before and after movement-in of fish. The sign test was applied to compare mortality medians of those matched paired time windows of each pond (Snedecor and Cochran, 1989).

Generalized estimating equations (GEE) with an exchangeable correlation structure within ponds was used to test whether the mean mortality before-exposure was equal to the mean after-exposure, using data across all ponds. Because the GEE was applied for marginal mean estimation with imbalanced clusters of fish mortalities in different ponds during the study period, I used an exchangeable correlation instead of using the other correlation structures, such as independence, autoregressive or unstructured (Bergsma et al., 2009).

4.3.4. Two-stage time series regression

A two-stage time-series regression (TSR) analysis (Bhaskaran et al., 2013) was used to assess risk factors of grass carp mortalities. All modeling steps were implemented in Stata 13 (Stata Corp., College Station, TX, USA).

In the first stage, the series of daily grass carp mortality counts from each pond were analyzed separately by generalized linear models. For these models, the distributional form, the modeling of temporal effects and the incorporation of autocorrelation were investigated in detail. Generally speaking, in order to obtain the most meaningful comparison across ponds (i.e., in the second stage of the modeling) it is preferable to use similar within-pond models for all ponds. On the other hand, computationally complex models may not be equally suited for all ponds, and in the most extreme cases, models that are too complex may fail to produce meaningful estimates within some ponds. Because excluding certain ponds from analysis due to computational problems would most likely lead to selection biases, our guiding principle for selecting appropriate within-pond models was to enable sufficient flexibility to capture the most important features of the data while allowing for estimation in all ponds. The impact of different choices for within-pond modeling was explored by sensitivity analysis.

The wide variability of within-pond counts (Table 4.1) led us to consider negative binomial instead of Poisson models. I adjusted for the population-at-risk by including a logarithmic fish total (*gctotal*) as an offset, and used maximum likelihood estimation and deviance residuals (as implemented in the glm command). Zero-inflated negative-binomial models with a constant zero-inflation proportion were compared to the negative-binomial models by Vuong's test (Vuong, 1989). The zero-inflated models require a more complex estimation procedure and do not allow for deviance residuals, so Pearson residuals (simple residuals divided by the standard deviation of observed counts) were used instead although deviance residuals are generally preferable (Bhaskaran et al., 2013).

Effects of time were explored by a smooth cubic spline function with varying numbers of knots (Bhaskaran et al., 2013). I initially evaluated between 2 and 9 knots, but due to convergence problems at the pond level when many knots were included, I restricted our models to splines with 5 and 6 knots.

Adjustment for autocorrelation was done by including both 1-week and 2-week lagged deviance and Pearson residual terms, as described above, in the predictive part of the model (Bhaskaran et al., 2013). Based on pretesting results of pond 23, I avoided dropping out of the 2 data points due to lagging the deviance residuals in the main model, namely number 7 and 8 in the series with mortality counts of 1 and 0, respectively. This method was also used in the sensitivity analysis. I also explored replacing the two residual terms by the previous day logarithmic outcome, following a practice in human disease TSR models (Imai et al., 2015).

In the second stage of each TSR model, the estimated coefficients for each predictor obtained from the analysis of each individual pond were combined and then subjected to a random-effects meta-analysis. Forest plots depicted the variability in predictor estimates across ponds, and their consistency was reflected in 95% confidence intervals (Jackson and Riley, 2010; Gasparrini et al., 2012; Gasparrini and Armstrong, 2013).

4.3.5. Sensitivity analysis

I compared the results from the two-stage TSR analysis to those based on different within-pond models (as discussed above); in addition, I also compared the results obtained for a multivariable analysis including all seven predictors simultaneously and separate analyses including one predictor at a time (together with other model terms). Based on descriptive and final model results, I also investigated the potential for confounding and correlation between some of the predictors, by comparing the results of the selected model to those without suitably chosen combinations of the predictors involved.

4.4. Results

4.4.1. Explorative descriptive analysis

4.4.1.1. Production information of study ponds

There were variations in start and finishing dates for the production cycle in the 14 ponds, with the earliest movement-in date in January 2013 (ponds 9 and 10), and latest movement-in date in April 2013 (pond 33) (Table 4.1). The mortality count pattern and the frequency of non-zero mortality days differed across ponds (Table 4.1). The 5 highest mortality counts were reported from ponds 10, 11, 12, 19, and 33. Between 32 and 80% of observations had zero mortality in each pond (Table 4.1), suggesting that at least some of the ponds had excessive zero mortality counts.

4.4.1.2. Descriptive analysis of temperature and management practices related to

Ambient temperature was considered a proxy for water temperature because the latter data source was incomplete. Based on fluctuation patterns of daily water and atmospheric temperature, we found that daily atmosphere temperatures were, overall, similar to the atmosphere temperature (Figure 4.1), indicating that it was reasonable to compute the *tmax06* by using atmosphere temperature.

Frequencies of management practices were summarized for each pond as shown in Table 4.2. For movements of fish, all 14 ponds experienced multiple movement-ins, but not all ponds were harvested multiple times. No movement-out of fish occurred in 3 ponds during the study period (ponds 21, 23, and 24), and movement-out of fish were recorded once for each of the 3 ponds (ponds 11, 22, and 33) (Table 4.2). For treatments of fish, most were applied at least once in each pond, except there were no antiparasitics treatments in ponds 13, 15, and 33. Applications of

Chinese medicine, probiotics, and water treatments were, overall, more frequently done than those of antibiotics and/or antiparasitics treatments across all ponds (Table 4.2). There was one exception, however, as fish in pond 11 were treated with antibiotics for about 37 days.

The simultaneous use of two groups of treatment, traditional Chinese medicine-probiotics (*ctpr7d*) and water quality improvement (*wimp3d*) was common in all the ponds (Fig 4.2). On the other hand, antibiotics and antiparasitics treatments were rarely combined with traditional Chinese medicine-probiotics, except in pond 11 (Fig 4.2).

Both treatments, traditional Chinese medicine-probiotics and water quality improvements, were likely to occur during those days with higher atmosphere temperature (Figs S4.1-2).

4.4.1.3. Mortalities before and after each management practices

Based on sign tests carried out for each pond, there was almost no difference between the before-event mortalities and after-event mortalities when each of 6 management practices was individually evaluated for each time window (Tables 4.3a and 4.3b).

All comparisons of GEE tests were not significant for both datasets with or without pond 33 ($p > 0.05$), indicating that after-intervention mortality was not always higher than before-intervention mortality (Tables 4.3c and 4.3d).

4.4.2. TSR modelling results

Pond 33 did not produce meaningful results for the first-stage TSR analysis; exploration of the data suggested this was due to irregularly-spaced missing data on fish mortality counts, and I therefore decided to exclude this pond from the TSR analysis. For pond 23, estimation in zero-inflated models was problematic, and for only two ponds (9 and 14) did the Vuong test suggest an improvement in fit with zero-inflation over ordinary negative-binomial models. For time modeling, a 5-knot spline was found to be the maximum number of knots for which negative-binomial models converged for all pond analyses. Including more knots than this caused the models to not converge for some ponds. I, therefore, focused limited our results for models which used either 5- or 6-knot splines. This number of knots has also been reported in other TSR studies on mortality counts (Bhaskaran et al., 2013). Summarizing these findings, I chose for our final model the following features: a negative binomial distribution (without zero-inflation), a 5-knot time spline, and two lagged deviance residual terms. The robustness of our results with this model to alternative model settings was explored by a sensitivity analysis, as discussed in section 5.4.3.

The results of applying our chosen model to 13 ponds (i.e., without pond 33) are shown in Table 4.4 and Figs 4.10-4.16. Four predictors had significant or close to significant impact on the incidence rate ratio (IRR) across all ponds, with the estimated mean and 95% confidence interval (CI) of: (1) delayed effects of moving in of fish (*mi2w*): 2.006 (95% CI, 1.504 to 2.676), (2) delayed effects of Chinese tradition medicine and probiotics (ctpr7d): 0.694 (95% CI, 0.566 to 0.852), (3) acute effects of water quality improvement (wimp3d): 1.236 (95% CI, 1.03 to 1.484) and (4) temperature (tmax06): 1.167 (95% CI, 1.063 to 1.281). In this main model, high levels of between-pond heterogeneity, sometimes referred to inconsistency (Higgins, 2003) , were only found for the coefficient estimation process of *tmax06* (I^2 =78.7%), and the estimates of the remaining 6 predictors were at moderate levels of heterogeneity with I^2 ranging from 45.3% to 59.8% (Figs 4.3-9).

Agreement between predicted and observed of daily mortality count was good across ponds, as shown in the plots of predictive and observed counts of carp mortality for each pond (Fig S5.9).

4.4.3. Sensitivity analysis of different model options

In the first part of our sensitivity analysis, I compared the results of our selected model to 7 alternative models with slightly different features, shown in Table 4.5. Two negative binomial models explored alternative ways of dealing with autocorrelation, by omitting the two deviance residual terms or by replacing them with a single lagged outcome term (Settings 2-3). One negative-binomial model explored the impact of increasing the number of spline knots from 5 to 6, thereby not including results from pond 23 (Setting 4). Two zero-inflated negative-binomial models were explored, with either 5 or 6 spline knots and Pearson residual terms (Settings 5-6), or replacing them with a single lagged outcome term (Setting 7). Finally, for the 5-spline knot model, with or without zero-inflation, estimation for each predictor on its own instead of in a multivariable model with 7 predictors was explored (Settings 8-9).

The results of the sensitivity analysis are shown for each of the 7 predictors individually in Figs 5.10-16. For most predictors, the sensitivity analyses agreed on the direction, approximate confidence interval range and overall significance (at $P<0.05$) of the coefficient; Exceptions were: the univariable models for *mi3d* and *cpr7d*, the two models based on 6 spline knots for *atbp7d*, and the model unadjusted for autocorrelation for *tmax06*. I will comment on each of these findings in turn. Additionally, most I^2-values of different all-predictor models were within the range of 25- 75%, indicating low to moderate levels of between-pond heterogeneity (Fig 4.17).

The two predictors *mi3d* and *mi2w* have overlapping time intervals for the entry of fish because the 3 days of *mi3d* are also included in the two-week interval of *mi2w* (Figs 4.10-11). In a univariable analysis, *mi3d* captures total mortality in the 3 days following movement, whereas in a multivariable model it captures additional mortality in those 3 days relative to the general change in mortality during two weeks after movement. The data show that the two-week effect is much stronger than the three-day effect, explaining the difference between univariable and multivariable effects for mi3d and indicating the former to be the most relevant.

The predictor *atbp7d* showed significant effects in the 2 models with 6 spline knots, with the IRR estimates of 1.611 (Settings 4) and 1.621 (Setting 7) respectively, contrasted with the non-significant effect of *atbp7d* estimated by models with 5 spline knots (Fig 4.13). This difference is essentially due to the exclusion of ponds 19 and 23 in the former models. In the 5-spline knot models without ponds 19 and 23, *atbp7d* is not significant ($P>0.05$), and its estimate was 1.362, different from those IRRs' estimated from all-variable models of 5 spline knots which ranged from 1.172-1.621 (Tables 4.4 and S4.1). Because there is no objective reason to exclude ponds 19 and 23 from analysis, I think the results for the 5-spline knot model are preferable.

The predictor *ctpr7d* was protective and significant in a multivariable model but showed no effect on its own (Fig 4.14), and whose inclusion strongly affects the coefficient for *ctpr7d*; hence the result of the multivariable analysis is the appropriate one to consider for *ctpr7d*. This can be explained as a confounding effect of temperature (tmax06), which was strongly associated to *ctpr7d* in some ponds where the treatments were essentially confined to high temperature ranges (Fig S4.1)

The impact of *wimp3d* varied substantially across sensitivity analyses, ranging in its estimated IRRs from 0.998 to 1.213, with the lowest estimates from univariable analyses (Fig 4.15). This appeared to be due less to a confounding effect of temperature (*tmax06*) than to a correlation with *ctpr7d* (Figs 4.2 and S4.2). Comparison of the group mean of *tmax06* indicated that water quality improvement was likely to happen on days with higher temperatures (Fig S4.2). Analyses with one or both of these predictors present showed that the overall significant conclusion for *ctpr7d* was not affected by the presence of wimp3d, while the reverse was not true. Additionally, among the multivariable analyses, both the number of spline knots and the distribution type appeared to impact the estimate to some degree. Because all changes in inference relative to the final model are towards the null, and there may also here be some selection bias from omitting ponds 19 and 23 (Table S4.1), a cautious conclusion would be that the results for the 5-spline knot model with all ponds are preferable. Considering these findings, I think it is fair to say that the results for *wimp3d* are inconclusive, but possibly suggestive of an increased risk.

There were some differences in estimates for *tmax06* across the models in our sensitivity analysis, although the range of estimates was relatively narrow, with IRRs from 1.11 to 1.19 (Fig 4.16). This was not unexpected because this predictor is strongly time-varying, and model choices for time modeling (number of spline knots, adjustment for autocorrelation) would therefore affect its estimate. On the other hand, the role of *tmax06* was to account for the biologically important impact of temperature and control for potential confounding effects on the management factors of primary interest, so the differences in its estimate and standard error are not necessarily of concern.

4.5. Discussion

This research is the first one applying TSR to modeling the risk factors of carp mortality based on logbook data collected as part of routine farm management. The 14-day lagged effect of movement-in of fish and temperature increase were significantly associated with increases in the mortality count of grass carp. In contrast, treatment with Chinese traditional medicine or probiotics was associated with reduction of grass carp mortalities.

Movement-in of fish. The relationship was evaluated between mortality and movement-in of fish within 14 days and the additive effect associated with fish movement-in within 3 days. The fact that I did not find a significant association with movement of fish 3 days prior suggests most movement on this farm did not exceed the biocapacity of the ponds. A significant increase was found in mortality within 14 days of introducing fish, which suggested that, on average, movements have negative impacts on ponds.

The introduction of new fish to a pond can introduce pathogens to the resident population. It can also adversely affect water quality if the pond was already heavily stocked, and new fish can stress the resident population by increasing competition for food and territory within the pond. The reverse is also possible. New fish can be exposed to novel pathogens from resident fish and be stressed from competition. Despite the issues that can arise from mixing fish populations, introductions of new fish into ponds, as well as partial harvests of populations, are common practices in carp aquaculture in earthen ponds. All-in-all-out farming strategies have been documented in several food animal production systems as effective in reduction of the likelihood of disease outbreaks (Rimstad et al., 2006; Cox and Pavic, 2010). However, all-in-all-out approaches might be difficult to apply in grass carp culture given its multiple movement-ins and harvests with the purpose of maximizing energy utilization of pond ecosystems (Lin and Peter, 1991).

Treatment with traditional Chinese medicine or probiotics. According to the original logbook records of the study ponds, traditional Chinese medicine treatment was usually administered together with probiotics and Vitamin C through feed. The delayed effect of this kind of treatment was found to be associated with reduced fish mortality. The commercial Chinese herb medicines contained the following main active components: Huang Qin (*Radix Scutellariae,* root of Baikal skullcap), Huang Bai (*Cortex Phellodendr*i, phellodendron bark), Da Qing Ye (*Folium Isatidis*, Isatis Leaf), Da Huang (Radix Et Rhizoma Rhei, Root And Rhizome of Sorrel Rhubarb), Da suan su (*Allantolin Allicin,* Allicine). The probiotics were commercial products containing mainly *Lactobacillus* sp., and other bacteria for which detailed information was not disclosed due to intellectual property issues. There have been studies addressing plant herbs as an alternative to antibiotics to treat fish disease (Jian and Wu, 2003; Bondad-Reantaso, 2012; Pandey et al., 2012; Guo et al., 2014). In the present study, this treatment appeared to help reduce the carp mortality. Further research should investigate the reasons why this treatment was associated with a reduction in mortality. However, antibiotic treatments were applied to their ponds on several occasions, so presumably the cause of mortality may have been infectious.

Water quality improvement. In this study, the chemicals used for water quality improvement mainly referred to povidone-iodine, calcium hypochlorite, copper sulfate, and chlorine dioxide. According to the anecdotal notes from fish farmers and fish vets in China during our surveys in 2014 (Chapters 2 and 3), compared with other health management practices, water quality treatment is more commonly adopted to prevent the occurrence of fish disease or reduce fish mortality. However, due to lack of fish disease diagnoses, farmers' decisions on water quality treatment rely on the guidance of fish health personnel and usually for prophylactic purposes, but without an accurate understanding of the role of water quality. In our study, this group treatment was potentially positively related to fish mortality instead of reducing the mortality. On the other hand, many endemic parasitic problems of finfish in ponds might compromise the integument of the fish and hence, a "blind" water quality treatment might exacerbate mortality instead of reducing it or simply not interrupt the "normal" increasing epidemic curve associated with the start of an infectious disease.

Water temperature. Water temperature level and changes are more likely to introduce cumulative chronic effect for the pond system (Pickering, 1998). Grass carp can tolerate temperatures from 0 to 33 ^{0}C. The upper lethal temperature range for grass carp is 33-41 ^{0}C with a mean critical thermal maximum of 39.3 ^{0}C (Chilton and Muoneke, 1992). However, under intensive pond aquaculture, the survival rates of grass carp were reported to be negatively related to an increase in ambient temperature (Song, 2012). Increases in water temperature might alter the toxicity of ammonia and cause the accumulation of ammonia and its metabolites in aquaculture systems, i.e. the nitrite intake (Alcaraz and Espina, 1995).

Time-series analysis offers various approaches to efficiently explain immediate and delayed effects when unknown or complex co-functioning factors exist, or when information is not available for detailed biological explanations of population health problems. Few time-series studies have been for aquatic animal health management, illustrating the difficulties in accurate measurement of mortality in the aquatic environment (Chang et al., 2007; Lessard et al., 2007; Connors, 2011). However, in warm-water aquaculture, application of such methods has not been reported for risk factor studies on farmed fish mortalities.

Distributional forms. I proposed a negative-binomial distribution as the main model for the within-pond analysis. Outcomes from different model options showed the robustness of our findings from the main model. Except for univariable models, the estimated coefficients were fairly consistent for most predictors between zero-inflated model and negative binomial, after controlling for other modeling components. For modeling count data with excess zeros, distribution form would more likely influence the standard errors than the estimated associations (Cox, 1983; Zeileis et al., 2007; Lee et al., 2011; Imai et al., 2015). In this study, the estimated coefficients for *tmax06* and *ctpr7d* from negative binomial and zero-inflated full models were generally consistent but the confidence intervals slightly varied. However, this was not the case for the *mi2w* coefficient, for which the estimates and confidence interval were more similar if the estimation processes used the same combination of autocorrelation terms and spline functions under different distribution forms. In other studies, it might be still worthwhile to explore whether the more elaborate model, i.e. zero-inflated model, would be helpful to substantially increase the validity of the analysis (Yang et al., 2010).

Smooth function of time. The cubic spline used in this study is one of natural smoothing spline functions which are useful to model non-linear association and capture autocorrelation in TSR analysis (Armstrong B., 2006). One need to choose the number of knots as "a reasonable compromise between controlling for confounding bias by unmeasured risk factors changing smoothly over time (compromised by too few knots) and retaining enough exposure contrast from which to estimate an association (compromised by too many knots)" (a personnel comment by Armstrong B.). Hence, the number of knots decided on for this study (nk=5) might be acknowledged as a reasonable choice based on judgement. However, for one predictor (*atbp7d*), I found that models using 6 knots instead of 5 changed the estimates from non-significant to significant.

It is well-known that the number of knots (also called as the degrees of freedom of splines) and placement might influence the flexibility of fit and also the estimated variances of the models (Katsouyanni K, 2003; Fleury et al., 2006; Newson, 2012; Bhaskaran et al., 2013). There is no uniformed criteria about the choices of the number of knots (National Research Council of Unite States, 2004; Bhaskaran et al., 2013), and decision could be data-driven or related to the specific data context targeted by TSR methods (Carder et al., 2005; Harrel, 2015; Imai et al., 2015). It somehow could remain controversial whether the spline function can cause over-adjustment bias (Box-Steffensmeier and Jones,2004; Imai et al., 2015). In this study, the shift of the estimates of *atbp7d* could be either due to the model choices or due to the removal of the ponds 19 and 23 for the model of 6 knots. Compared with other estimates generated by the full models, the interpretation for the effect of water quality improvement might be less certain than the effects of all the other predictors. Furthermore, the model with number of knots of 5 was able to avoid the loss of the ponds 19 and 23 data from the data analysis due to convergence problem.

Autocorrelation. One of the autocorrelation terms used in this study is the log term of the mortality count of the previous day (Peng, 2006; Imai et al., 2015), which is less commonly used than the approach of using lagged residuals in TSR, but can be justified mathematically for infectious diseases, might help with non-convergence problems (Imai and Hashizume, 2015). In this study, this autocorrelation approach was found to have a limited effect on the results. It might not be necessary to make judgements about using the autocorrelation approach only based on whether mortality is to be caused by infectious disease (Imai and Hashizume, 2015).

A few issues with our particular dataset might limit our interpretations of the model estimates. First, I had one pond that was not similar to the others and could not be modeled like the others (pond33). This pond had higher than average zero counts and sudden increases in mortality followed or preceded by missing data. I had to exclude this pond in order to complete this study. Secondly, the variable *tmax06* had the first 6 observations missing for each pond, so these observations were not included in the models. Since mortality immediately after initial movement-in was not our main interest, I was not too concerned that this time period was missing from our study. Although there are no previous carp mortality studies to support this, the data for the first week after movement-in are sometimes excluded in studies of salmon mortalities. Loss of the first few observations might be common in TSR, but less of a limitation if the early days of the series are not of special interest. Thirdly, correlation between treatment predictors and *tmax06* was found to be high in most ponds. The use of Chinese traditional medicine and probiotics were found to be often used simultaneously with water quality improvement, which indicated the correlation of the two predictors: *atbp7d* and *ctpr7d*. Furthermore, there could be measurement bias due to count of daily dead fish and misclassification bias originated from treatment methods. Detailed exploration on bias was not included in this study, but could be indicative for future studies.

As only a single farm was represented in this study, the external validity of this study is limited. Results may not able to apply to farms in other geographical locations or managed under different circumstances. However, the methodologies of TSR modeling will be useful for risk factor studies of grass carp mortality on other farms. This study demonstrated how to utilize fish farm logbook data in time-series studies of mortality and hence, it showed the feasibility of exploring evidence-based methods for aquatic health management in China.

4.6. Conclusions

To our knowledge, this is the first application of TSR to a risk factor study of daily mortalities of warmwater finfish. Results of the main negative-binomial regression model using two-stage analyses indicated that movement of fish into ponds, water quality improvement and temperature increases might have negative effects on the survival of grass carp, while treatments using traditional Chinese medicine and probiotics might be effective in reducing grass carp mortality. Although generalizability of these findings to other settings should be made with caution, the methods and modeling undertaken revealed the value of daily record-keeping on small-scale farms. This was the first time such records have been analyzed, for Chinese carp aquaculture, and this study highlights for the industry the importance and usefulness of this type of data.

4.7. Acknowledgements

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Table 4.1 Stocking time, final date of production, and grass carp mortality counts summarized for each pond.

Stocking date	Pond	Final record	All mortality counts						Non-zero mortality counts				
			Min	Max	Mean	Median	SD	Skewness	Event frequency*[a]	Mean	Median	SD	Skewness
1/14/2013	9	6/23/2013	0	174	11.9	1	29	3.2	0.547	21.8	3	36.4	2.2
1/14/2013	10	9/24/2013	0	295	7.2	0	28.5	8	0.449	16.1	3	40.9	5.4
1/15/2013	11	9/24/2013	0	1620	84.7	11	177.4	4.5	0.569	148.8	73	214.1	3.6
1/16/2013	12	9/24/2013	0	300	9.2	0	28.7	6.3	0.44	20.9	6	40.5	4.3
1/17/2013	13	8/30/2013	0	73	4.4	2	8.3	4.7	0.681	6.4	3	9.4	4.1
1/19/2013	14	9/24/2013	0	63	9	1	13.3	1.7	0.522	17.3	15.5	13.9	1
1/17/2013	15	8/30/2013	0	76	4.7	2	9.7	4.2	0.633	7.5	4	11.4	3.4
3/14/2013	19	8/30/2013	0	411	11.8	0	50.5	5.8	0.465	25.4	3	71.9	3.8
3/15/2013	20	9/24/2013	0	81	2.6	0	8.8	6.3	0.345	7.6	3	13.7	3.8
3/26/2013	21	8/30/2013	0	95	10	1	20.8	2.5	0.551	18.1	5	25.3	1.6
3/25/2013	22	9/24/2013	0	68	3.7	0	8.9	4.9	0.495	7.5	4	11.5	3.6
3/26/2013	23	8/30/2013	0	212	11	0	35.2	4.8	0.43	25.6	8	50.3	3
3/25/2013	24	9/24/2013	0	41	5.4	1	7.9	1.7	0.522	10.4	9	8.3	1
4/29/2013	33	9/24/2013	0	870	38.9	0	111.9	4.1	0.201	193	144	182.1	1.8
	Total		0	1620	16	0	66.9	10.9	0.498	32.2	6	92.1	7.9

Note: *[a] Event denoted a day with mortality of grass carp more than zero.

Table 4.2 Frequencies of management variables: movements and treatments of fish.

	Movement of fish		Treatment of fish				
Pond	Stocking	Harvest	Antibiotics	Antiparasitics	CTM	Probiotics	Water improvement
9	9	5	15	2	26	14	35
10	7	3	7	3	30	24	41
11	3	1	37	2	58	15	37
12	7	9	3	3	25	15	36
13	5	3	5	0	37	17	41
14	6	5	1	6	31	19	40
15	5	4	2	0	28	17	39
19	6	6	5	3	15	18	21
20	6	3	3	2	14	19	24
21	4	0	0	2	20	17	31
22	4	1	2	2	27	21	36
23	4	0	11	3	17	14	34
24	4	0	2	2	35	26	40
33	8	1	13	0	30	14	40

Table 4.3a Nonparametric paired comparison between mortalities (x10^4) of 3 or 14 days pre-movement and those of 3 or 14 days post-movement in each pond

	3-day window of movement-in						**14-day window of movement-in**						**3-day window of movement-out**					
	Before		After		Sign test		Before		After		Sign test		Before		After		Sign test	
Pond	N	mort3db	N	mort3da	p1*[a]	p2*[b]	N	mort14db	N	mort14da	p1	p2	N	mort3db	N	mort3da	p1	p2
9	7	0	9	1.09	1.00	0.03	5	10.88	6	11.96	0.50	0.81	5	161.63	2	230.29	0.75	0.75
10	5	0	7	0	0.75	0.75	4	4.12	5	31.38	0.69	0.69	3	73.51	3	264.23	1.00	1.00
11	2	10.18	3	0.29	0.75	0.75	2	93.43	2	441.74	0.75	0.75	1	0	1	0	1.00	1.00
12	4	0	7	0	1.00	0.50	2	0.38	4	0.75	1.00	0.25	9	1.13	9	19.43	0.91	0.25
13	4	6.13	5	6.65	0.94	0.31	3	27.86	4	28.38	0.50	0.88	3	77.68	3	69.14	0.13	1.00
14	4	0	6	0	0.88	0.50	2	1.8	4	1.11	0.25	1.00	5	0	4	20.26	1.00	0.13
15	4	6.99	5	2.87	0.50	0.88	3	25.96	4	29.29	0.88	0.50	4	34.1	4	65.93	0.94	0.31
19	4	0	6	30.94	0.75	0.75	3	52.22	3	0	0.50	0.88	6	24.36	6	7.35	1.00	0.13
20	4	1.67	6	19.87	0.69	0.69	3	73.14	3	3.33	0.50	0.88	3	3.53	3	29	1.00	0.50
21	3	1.85	4	0.93	0.88	0.50	2	149.86	3	4.33	1.00	0.25	0					
22	3	3.84	4	1.28	0.50	0.88	1	93.32	3	3.84	1.00	0.50	1	66.42	1	86.22	1.00	0.50
23	3	2.05	4	1.36	0.75	0.75	2	24.62	3	1.37	1.00	0.50	0					
24	3	6.13	4	2.68	0.50	0.88	1	122.31	3	5.36	1.00	0.50	0					
33	6	0	8	0	1.00	0.50	5	0	5	0	0.88	0.50	1	509.76		0	1.00	1.00

Note *[a, b]: One-sided sign test, with alternative hypotheses that probability of post-movement mortality was larger (or smaller than) pre-movement mortality, respectively. For examples, if p1<0.05, the null hypothesis of equal probability of larger and smaller post-movement probability would be rejected in favor of a larger post-movement probability.

Table 4.3b Nonparametric paired comparison of between the median mortalities (x10^4) of 3 or 7 days pre-treatment and those of 3 or 7 days post-treatment in each pond.

	Antibiotics-antiparasitics						Chinese traditional medicine-probiotics						Water quality improvement					
	Before		After		Sign test		Before		After		Sign test		Before		After		Sign test	
Pond	N	mort7db	N	mort7da	p1*[a]	p2*[b]	N	mort7db	N	mort7da	p1	p2	N	mort3db	N	mort3da	p1	p2
9	17	141.83	17	341.72	0.99	0.02	37	15.29	37	14.25	0.95	0.09	35	8.74	35	6.59	0.70	0.43
10	10	189.07	10	1692.86	0.83	0.38	44	15.77	44	11.47	0.56	0.56	41	5.11	41	5.09	0.06	0.97
11	39	341.55	39	233.96	0.09	0.95	63	69.95	63	85.77	0.05	0.97	37	4.41	37	3.88	0.35	0.78
12	6	35.62	6	42.89	0.98	0.11	37	41.55	37	121.91	1.00	0.00	36	12.51	36	19.44	0.99	0.02
13	3	8.9	5	11.13	1.00	0.13	38	27.29	39	21.50	0.01	1.00	41	8.92	40	6.79	0.56	0.56
14	7	134.62	7	109.78	0.77	0.50	40	83.1	40	86.63	0.68	0.44	40	18.65	40	24.26	0.68	0.44
15	0		2	1.91	1.00	1.00	32	24.78	33	25.45	0.93	0.14	39	7.92	38	6.38	0.25	0.84
19	8	18.32	8	12.95	0.36	0.86	26	19.39	25	16.22	0.34	0.80	21	8.08	20	9.72	0.89	0.23
20	5	46.73	5	484.02	1.00	0.03	26	42.55	26	46.74	0.92	0.15	24	8.83	24	1.76	0.12	0.95
21	2	232.23	2	76.85	0.25	1.00	29	24.79	31	16.71	0.64	0.50	31	9.28	31	9.90	0.57	0.57
22	4	93.10	4	96.17	0.94	0.31	35	21.87	36	23.18	0.09	0.96	36	7.73	36	9.66	0.16	0.91
23	13	53.47	13	44.52	0.50	0.71	23	3.41	25	1.37	<0.01	1.00	34	11.58	34	9.58	0.01	0.99
24	4	52.14	4	79.81	0.69	0.69	39	59.26	40	40.71	0.05	0.97	40	23.05	40	14.2	0.17	0.90
33	13	1268.17	13	1164.14	0.50	0.71	35	6.12	35	0	0.41	0.75	40	0	40	0	0.40	0.77

Note *[a, b]: One-sided sign test, with alternative hypotheses that probability of post-treatment mortality was larger (or smaller than) pre-treatment mortality, respectively. For examples, if p1<0.05, the null hypothesis of equal probability of larger and smaller post-treatment probability would be rejected in favor of a larger post-treatment probability.

Table 4.3c Summary of GEE results applied to full datasets when one of the following interventions took place.

Interventions and time window	Estimated odds*[a]	95% Confidence interval	P value
3 days before and after movement-in of fish	1.633	(0.826, 3.349)	0.154
14 days before and after movement-in of fish	0.996	(0.635, 1.469)	0.872
3 days before and after movement-out of fish	1.984	(0.630, 6.249)	0.291
7 days before and after treatment with antibiotics or antiparasitics	1.383	(0.848, 2.258)	0.194
7 days before and after treatment with CTM or probiotics	0.922	(0.922, 0.657)	0.637
7 days before and after treatment with water improvement chemicals	0.859	(0.859, 0.681)	0.216

Note: *[a] Odds referred to the probability of after-intervention mortality being larger than before-intervention mortality within the given time window divided by the probability of after-intervention mortality not being larger than before-intervention mortality within the given time window

Table 4.3d Summary of GEE results applied to partial datasets with the removal of Pond33 when one of the following interventions took place.

Interventions and time window	Estimated odds*[a]	95% Confidence interval	P value
3 days before and after movement-in of fish	0.862	(0.676, 1.101)	0.234
14 days before and after movement-in of fish	0.937	(0.609, 1.443)	0.769
3 days before and after movement-out of fish	1.984	(0.630, 6.246)	0.242
7 days before and after treatment with antibiotics or antiparasitics	1.481	(0.857, 2.560)	0.159
7 days before and after treatment with CTM or probiotics	0.930	(0.647, 1.334)	0.691
7 days before and after treatment with water improvement chemicals	0.862	(0.676, 1.101)	0.234

Note: *[a] Odds referred to the probability of after-intervention mortality being larger than before-intervention mortality within the given time window divided by the probability of after-intervention mortality not being larger than before-intervention mortality within the given time window.

Table 4.4 Estimated mean and 95% confidence intervals of each predictor calculated from the main model (indicated as the model of 1. nk5 lag2 in Table 4.5)

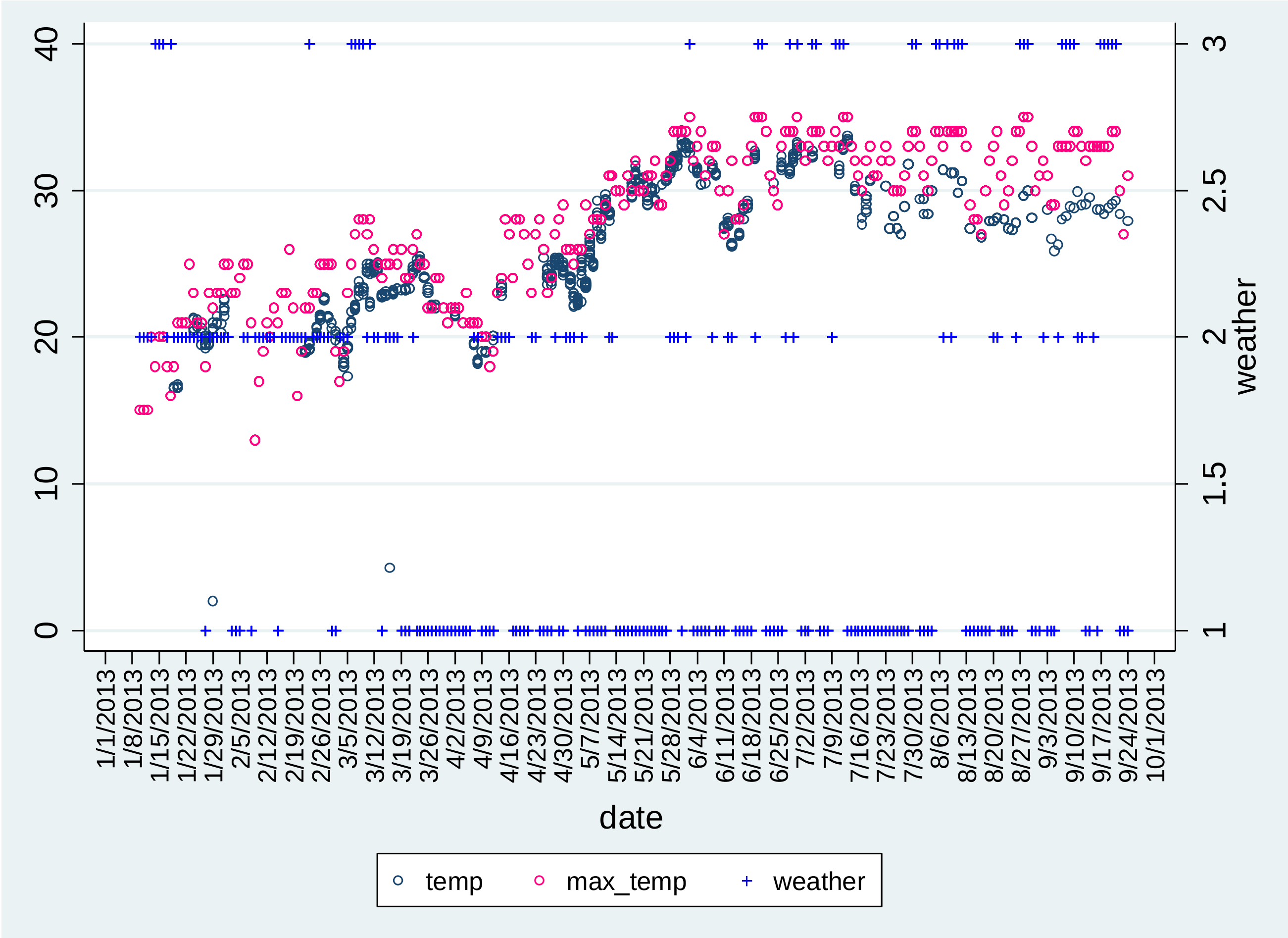
			95% Confidence interval	
Predictor variables and effects evaluated	**Coefficient**	**P**	**Lower**	**Upper**
Movement of fish within previous 3 days (*mi3d*=1)	0.834	0.458	0.571	1.346
Movement in of fish within previous 14 days (*mi2w*=1)	2.006	<0.001	1.504	2.676
Movement out of fish within previous 3 days (*mo3d*=1)	1.369	0.221	0.828	2.262
Treatment of antibiotics or antiparasitics (*atbp7d*=1)	1.282	0.077	0.973	1.69
Treatment of TCM or antibiotics (*ctpr3d*=1)	0.694	<0.001	0.566	0.852
Acute effect of water treatment (*wimp3d*=1)	1.213	0.058	0.993	1.481
Temperature of previous week increase by 1 ^{0}C (*tmax06*)	1.167	0.001	1.063	1.281

Table 4.5 Sensitivity analyses: TSR models for full models and univariable models substituted with different distributional forms, number of knots in spline, and autocorrelation options.

TSR Model abbreviation	Distributional form	Number of knots	Auto correlation term	Predictors included	Ponds analyzed
1. nb nk5 lag2 allvar	negative binomial	5	Deviance residual	All predictors	all 13 ponds*[a]
2. nb nk5 noAC allvar	negative binomial	5	No residual	All predictors	all 13 ponds
3. nb nk5 logpre allvar	negative binomial	5	logpregcdeath	All predictors	all 13 ponds
4. nb nk6 lag2 allvar	negative binomial	6	Deviance residual	All predictors	all 13 ponds except pond 23
5. zinb nk5 lag2 allvar	zero-inflated negative binomial	5	Pearson residual	All predictors	all 13 ponds
6. zinb nk5 logpre allvar	zero-inflated negative binomial	5	logpregcdeath	All predictors	all 13 ponds
7. zinb nk6 lag2 allvar	zero-inflated negative binomial	6	Pearson residual	All predictors	all 13 ponds except pond 23
8. nb nk5 lag2 univar	negative binomial	5	Deviance residual	Univariable	all 13 ponds
9. zinb nk5 lag2 univar	zero-inflated negative binomial	5	Pearson residual	Univariable	all 13 ponds

Note: *[a] Among the originally recorded 14 ponds in Table 4.1, all the other 13ponds were included in the time series analysis except pond 33.

Fig 4.1 Fluctuation of atmosphere temperature and water temperature recorded.

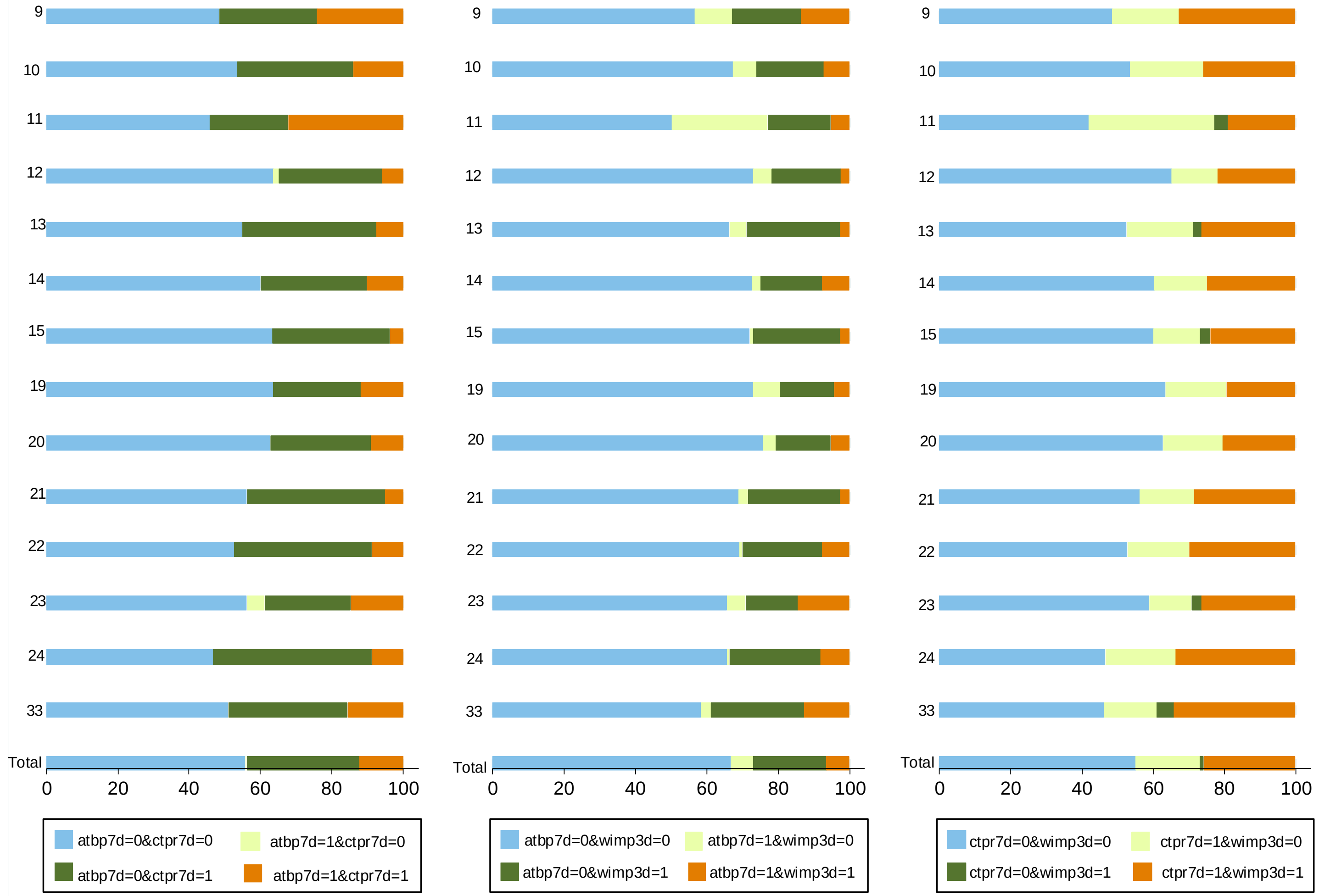


Note: 1. *temp* denoted water temperature measurement records in the data. Variation of water temperature among different ponds was assumed to be negligible.

2. *max_temp* denoted atmosphere temperature from online weather historical record for the study area.

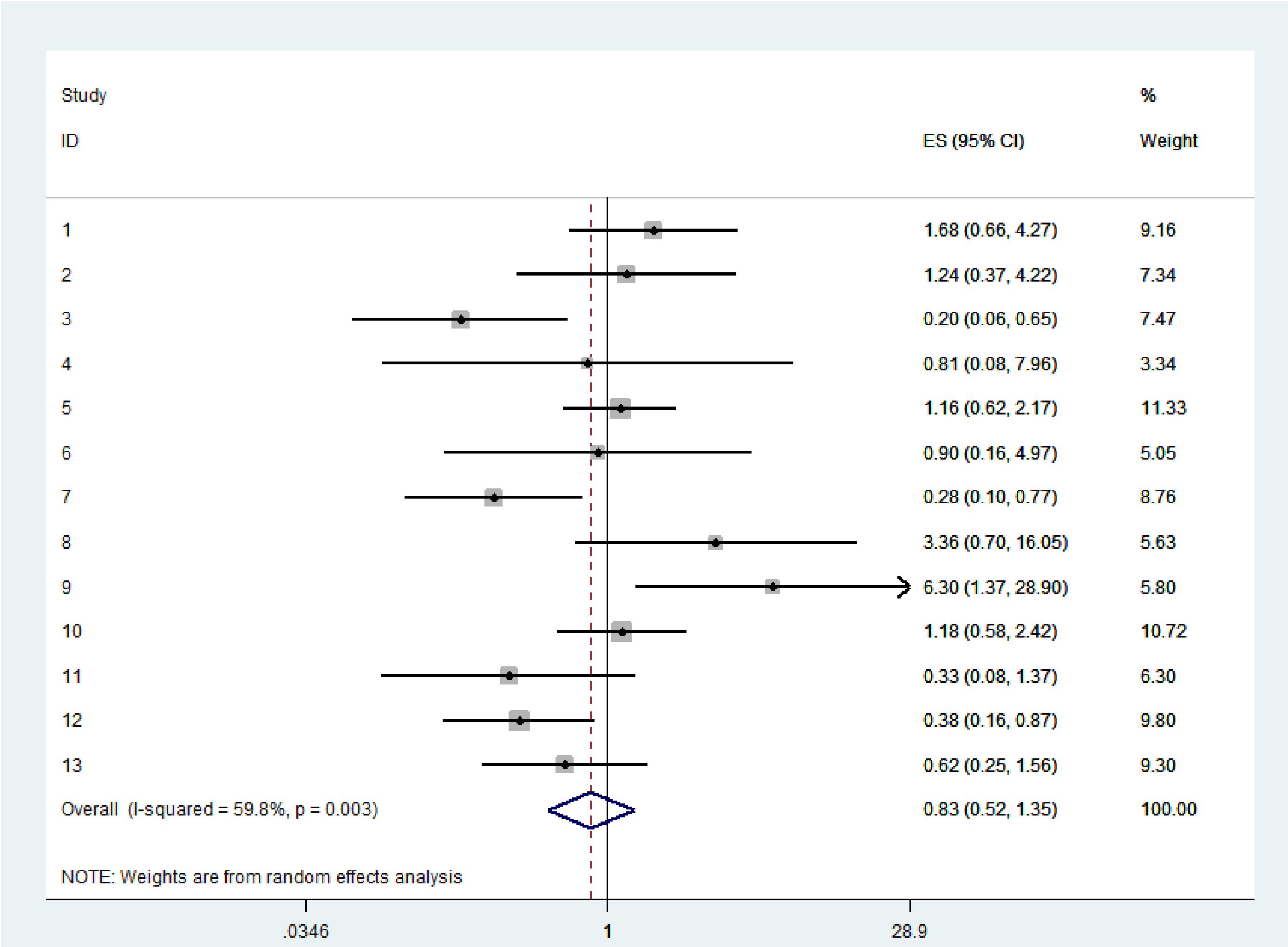
3. *weather* denotes sunny with the value of 3, cloudy with the value of 2 and rain with the value of 1.

Fig 4.2 Frequencies proportion about whether each of the 3 treatments predictors has occurred simultaneously with any of the other 2 treatments for the 14 ponds.



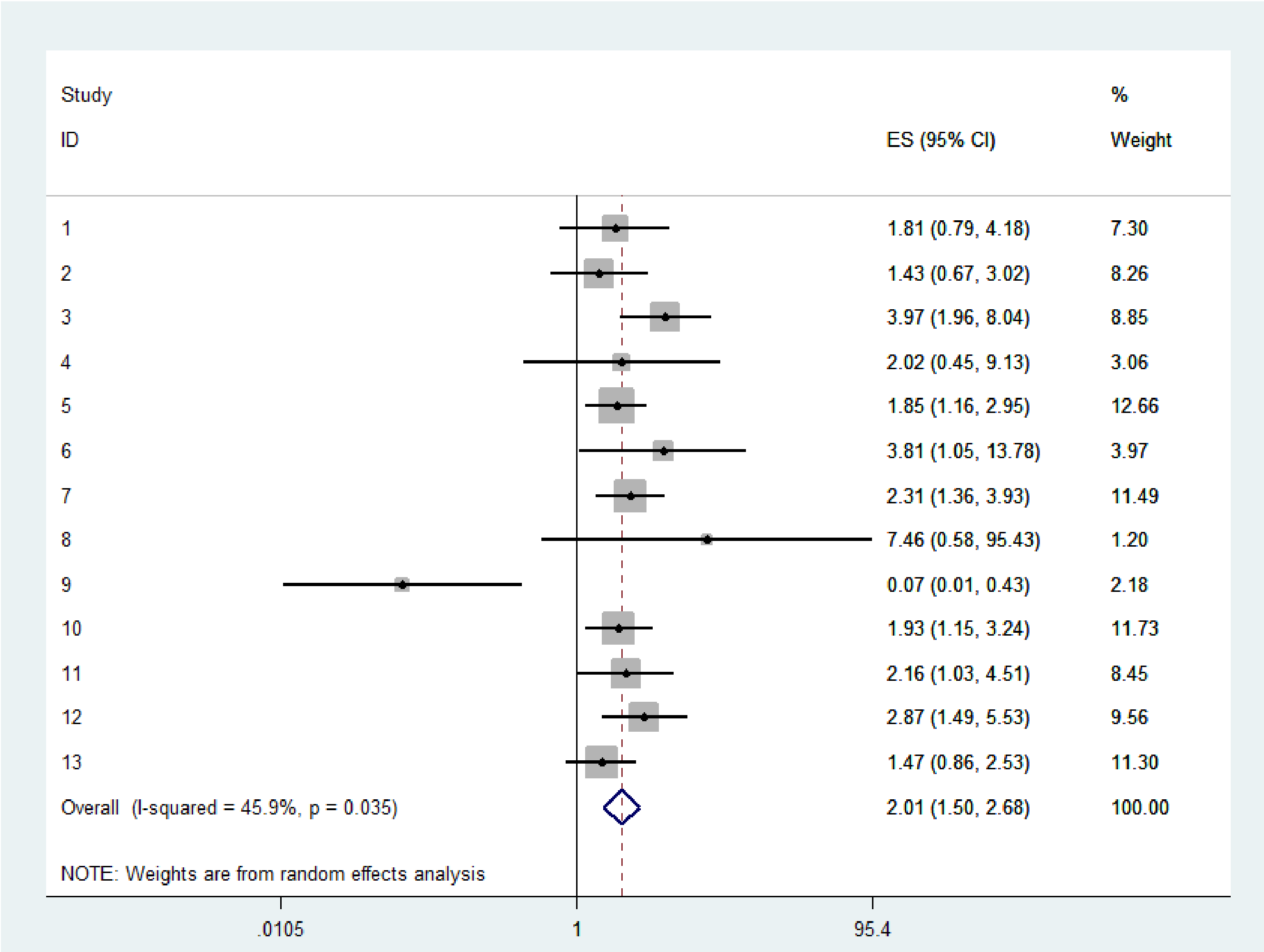
S

Fig 4.3 Forest plots for the random effect estimation of *mi3d* by the main model across the 13 ponds[*a].



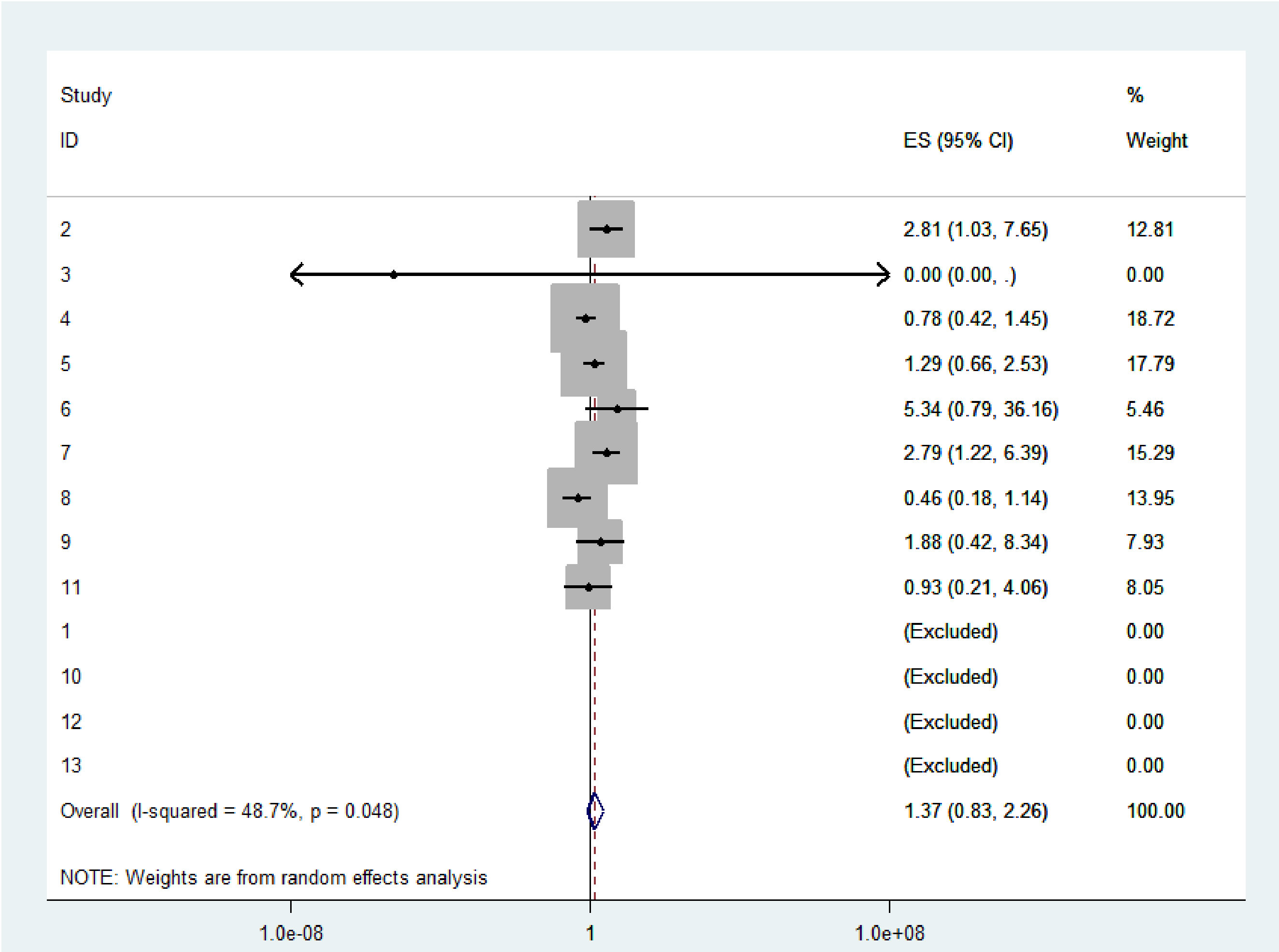
Note: [*a] The 13 ponds are pond 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24, correspondingly listed in ascending order. Pond 33 was omitted.

Fig 4.4 Forest plots for the random effect estimation of mi2w by the main model across the 13 ponds*[a].



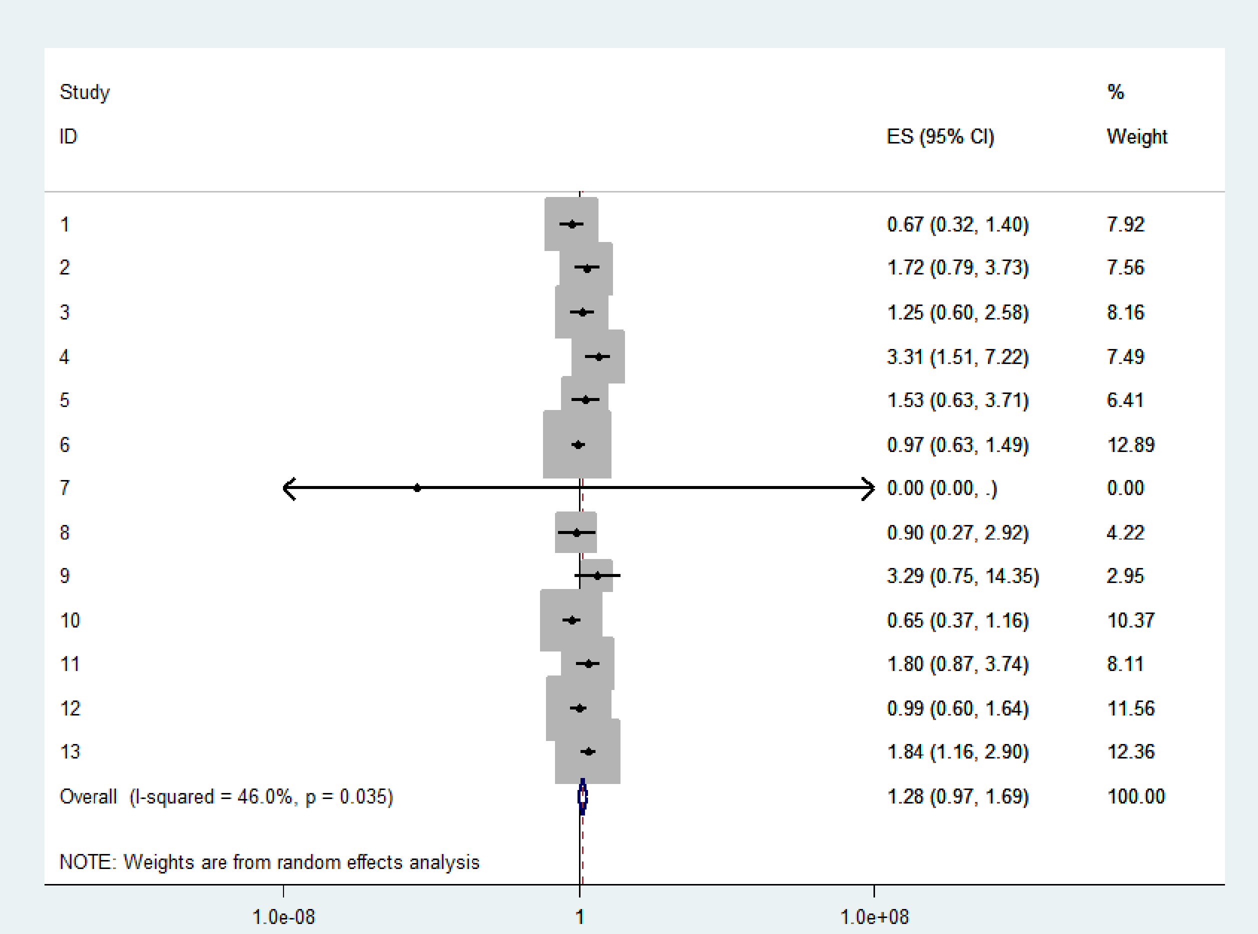
Note: *[a] The 13 ponds are pond 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24, correspondingly listed in ascending order. Pond 33 was omitted.

Fig 4.5 Forest plots for the random effect estimation of *mo3dm* by the main model across the 13 ponds*[a].



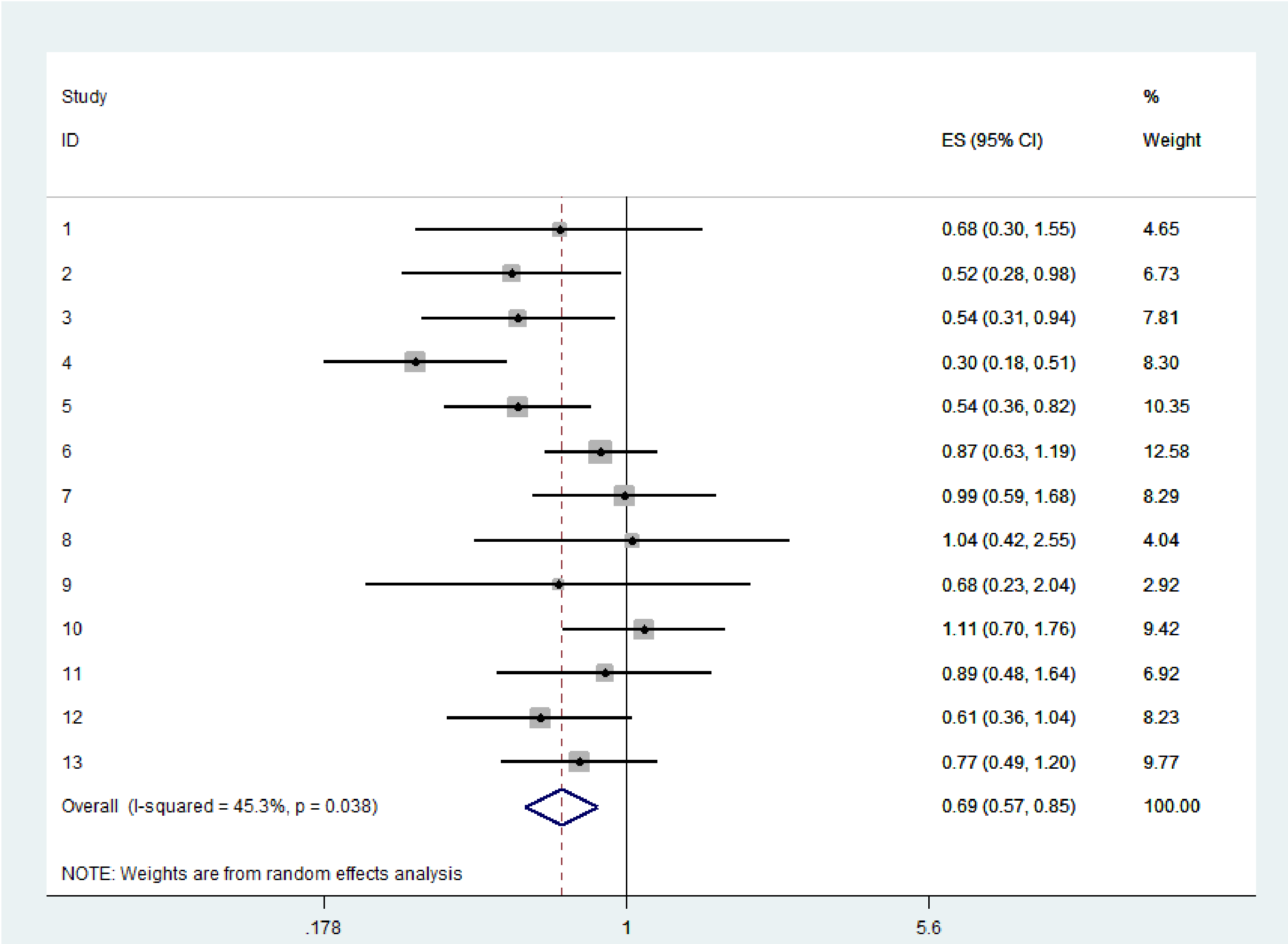
Note: *[a] The 13 ponds are pond 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24, correspondingly listed in ascending order. Pond 33 was omitted.

Fig 4.6 Forest plots for the estimation of *atbp7d* by the main model across the 13 ponds*[a].



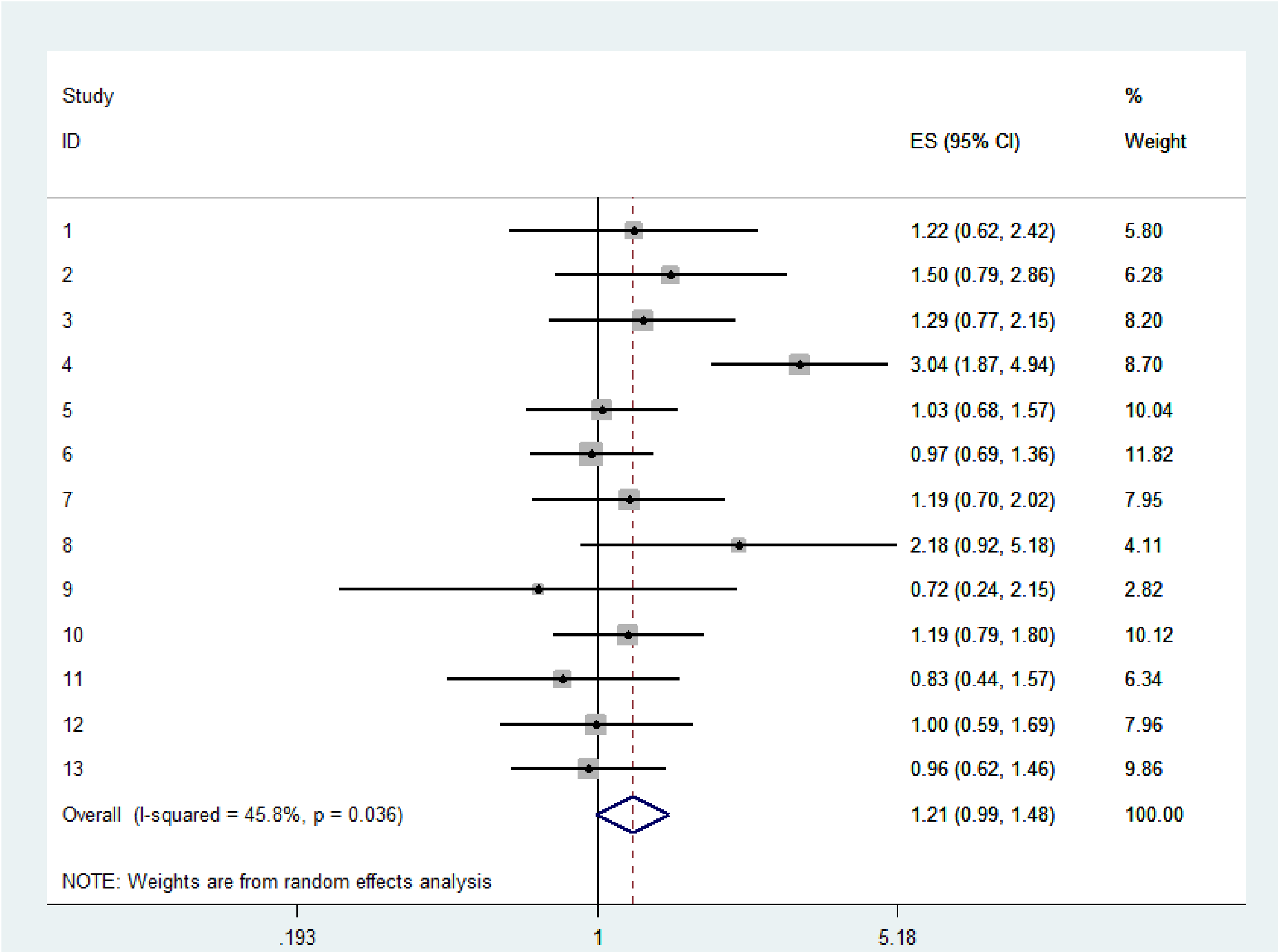
Note: *[a] The 13 ponds are pond 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24, correspondingly listed in ascending order. Pond 33 was omitted.

Fig 4.7 Forest plots for the estimation of *ctpr7d* by the main model across the 13 ponds*[a].



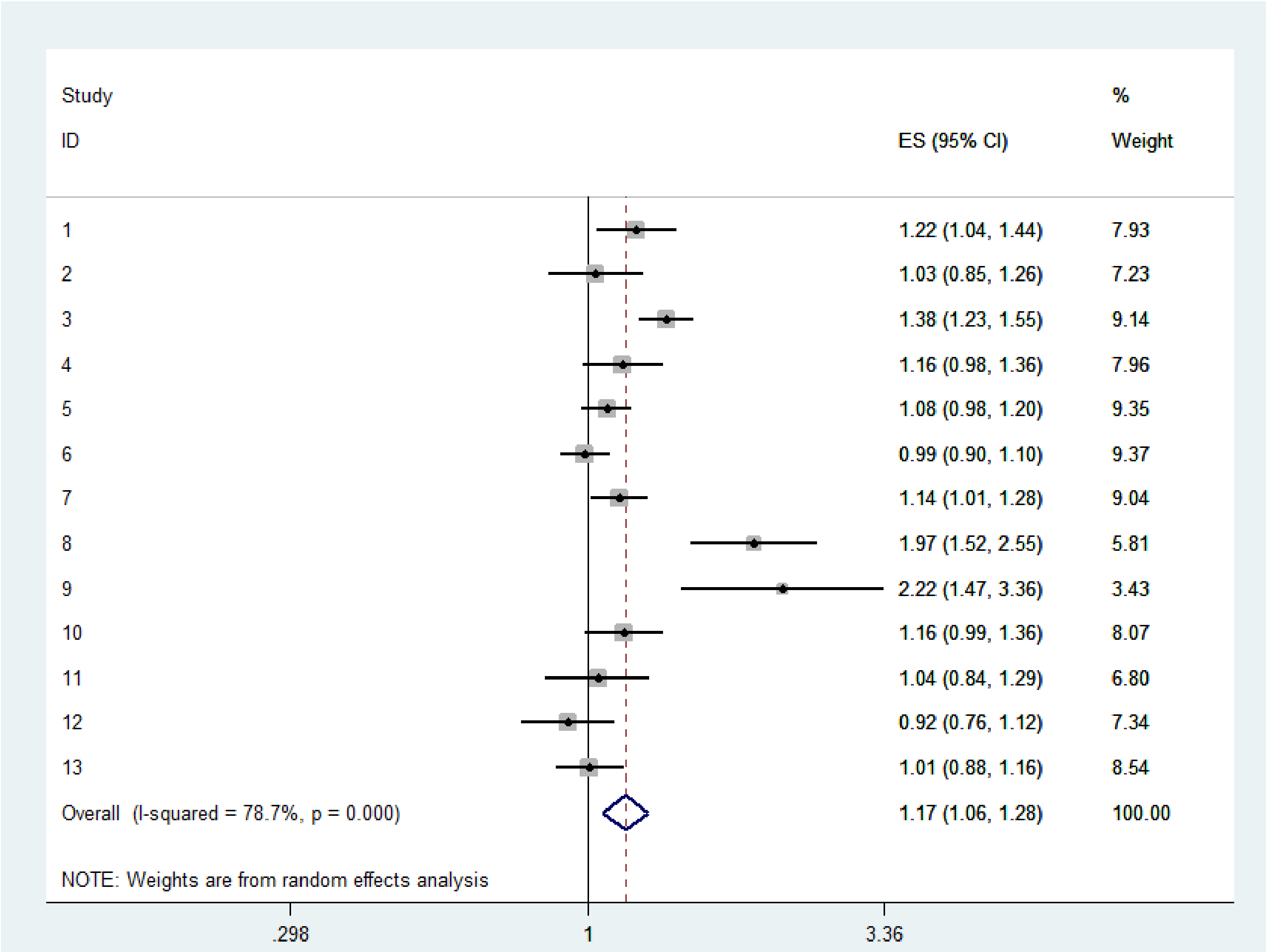
Note: *[a] The 13 ponds are pond 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24, correspondingly listed in ascending order. Pond 33 was omitted.

Fig 4.8 Forest plots for the estimation of *wimp3d* by the main model across the 13 ponds*[a].



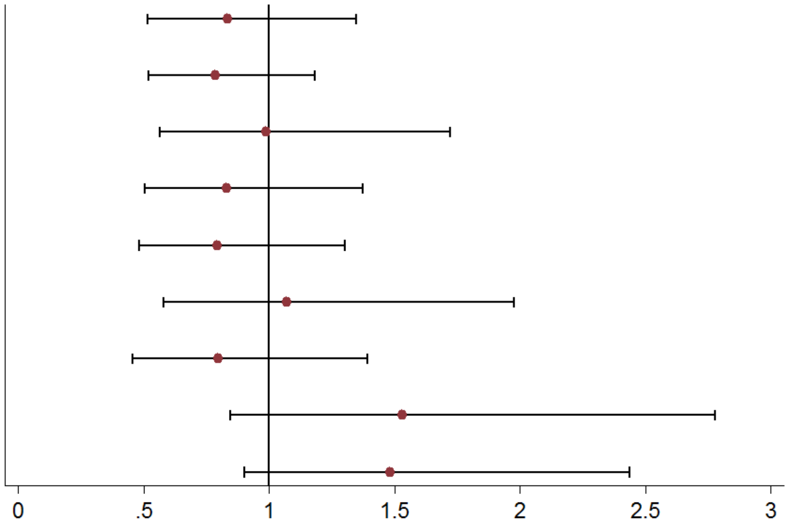
Note: *[a] The 13 ponds are pond 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24, correspondingly listed in ascending order. Pond 33 was omitted.

Fig 4.9 Forest plots for the estimation of *tmax06* by the main model across the 13 ponds*[a].



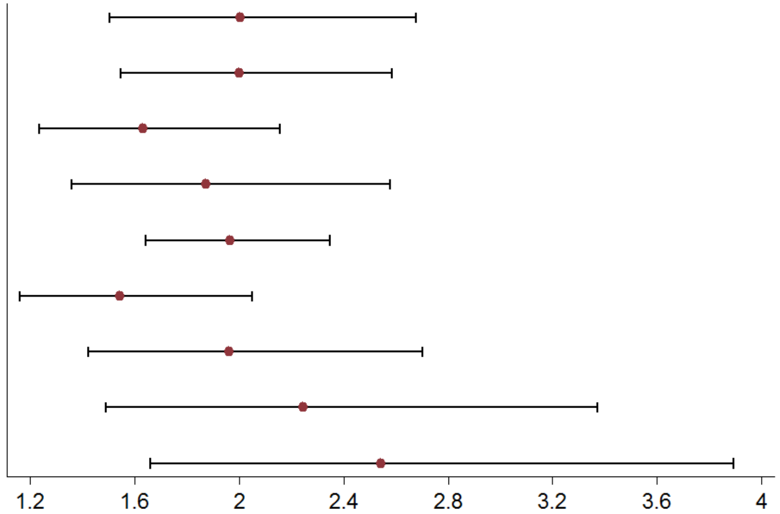
Note: *[a] The 13 ponds are pond 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24, correspondingly listed in ascending order. Pond 33 was omitted.

Fig 4.10 Sensitivity analysis for estimation of incidence rate ratio (IRR) of *mi3d* using all-predictor and univariable models.



Model	P	IRR	95% CI	Heterogeneity P	I^2
1. nb nk5 lag2 allvar	0.458	0.834	(0.517, 1.346)	0.003	59.80%
2. nb nk5 noAC allvar	0.249	0.786	(0.521, 1.184)	0.05	42.90%
3. nb nk5 logpre allvar	0.6833	0.986	(0.565, 1.721)	<0.001	71.00%
4. nb nk6 lag2 allvar	0.472	0.832	(0.505, 1.373)	0.013	55.20%
5. zinb nk5 lag2 allvar	0.36	0.793	(0.482, 1.303)	<0.001	67.80%
6. zinb nk5 logpre allvar	0.827	1.071	(0.581, 1.975)	<0.001	79.60%
7. zinb nk6 lag2 all var	0.425	0.797	(0.456, 1.393)	<0.001	68.70%
8. nb nk5 lag2 univar	0.161	1.531	(0.844, 2.776)	<0.001	79.60%
9. zinb nk5 lag2 univar	0.121	1.481	(0.901, 2.435)	<0.001	72.50%

Fig 4.11 Sensitivity analysis for estimation of incidence rate ratio (IRR) of *mi2w* using all-predictor and univariable models.



Model	P	IRR	95% CI		Heterogeneity P	I^2
1. nb nk5 lag2 allvar	<0.001	2.006	(1.504,	2.676)	0.035	45.90%
2. nb nk5 noAC allvar	<0.001	2.000	(1.547,	2.585)	0.151	29.30%
3. nb nk5 logpre allvar	0.001	1.633	(1.237,	2.157)	0.069	39.60%
4. nb nk6 lag2 allvar	<0.001	1.873	(1.361,	2.577)	0.031	49.40%
5. zinb nk5 lag2 allvar	<0.001	1.966	(1.645,	2.348)	0.494	0.00%
6. zinb nk5 logpre allvar	0.003	1.545	(1.163,	2.051)	0.022	49.60%
7. zinb nk6 lag2 all var	<0.001	1.962	(1.423,	2.704)	0.010	56.70%
8. nb nk5 lag2 univar	<0.001	2.244	(1.493,	3.371)	<0.001	76.90%
9. zinb nk5 lag2 univar	<0.001	2.544	(1.663,	3.892)	<0.001	81.60%

Fig 4.12 Sensitivity analysis for estimation of incidence rate ratio (IRR) of *mo3dm* using all-predictor and univariable models.

Model	**P**	**IRR**	**95% CI**	**Heterogeneity P**	**I^2**
1. nb nk5 lag2 allvar	0.221	1.368	(0.828, 2.262)	0.048	48.70%
2. nb nk5 noAC allvar	0.146	1.369	(0.896, 2.091)	0.212	26.00%
3. nb nk5 logpre allvar	0.077	1.447	(0.960, 2.181)	0.213	25.90%
4. nb nk6 lag2 allvar	0.26	1.339	(0.806, 2.227)	0.108	40.70%
5. zinb nk5 lag2 allvar	0.195	1.422	(0.835, 2.422)	0.018	56.90%
6. zinb nk5 logpre allvar	0.078	1.46	(0.958, 2.225)	0.146	34.00%
7. zinb nk6 lag2 all var	0.272	1.372	(0.780, 2.415)	0.026	55.90%
8. nb nk5 lag2 univar	0.155	1.458	(0.867, 2.450)	0.048	48.80%
9. zinb nk5 lag2 univar	0.139	1.526	(0.872, 2.670)	0.013	58.70%

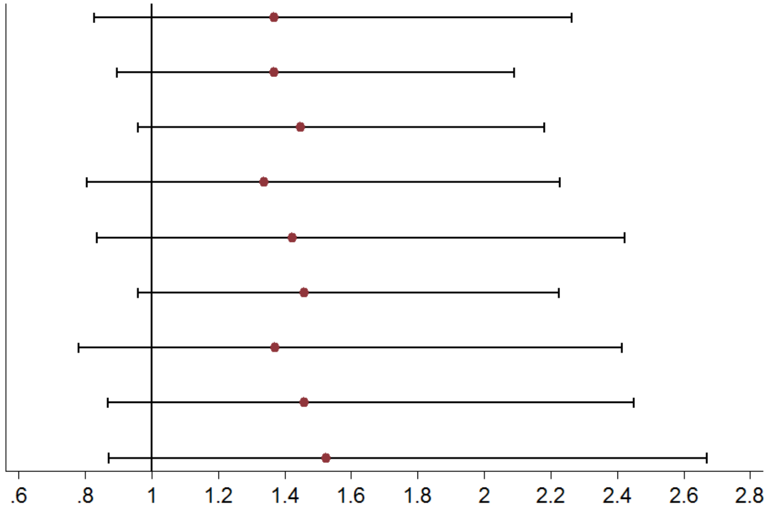


Fig 4.13 Sensitivity analysis for estimation of incidence rate ratio (IRR) of *atpbp7d* using all-predictor and univariable models.

Model	P	IRR	95% CI	Heterogeneity P	I^2
1. nb nk5 lag2 allvar	0.077	1.282	(0.973, 1.690)	0.035	46.00%
2. nb nk5 noAC allvar	0.070	1.258	(0.981, 1.613)	0.16	28.30%
3. nb nk5 logpre allvar	0.206	1.172	(0.916, 1.498)	0.127	32.00%
4. nb nk6 lag2 allvar	0.003	1.611	(1.180, 2.200)	0.041	47.30%
5. zinb nk5 lag2 allvar	0.073	1.342	(0.974, 1.849)	< 0.001	70.00%
6. zinb nk5 logpre allvar	0.170	1.2	(0.925, 1.556)	0.012	53.30%
7. zinb nk6 lag2 all var	0.015	1.621	(1.097, 2.394)	< 0.001	74.90%
8. nb nk5 lag2 univar	0.690	1.084	(0.730, 1.609)	< 0.001	82.80%
9. zinb nk5 lag2 univar	0.725	1.073	(0.725, 1.586)	< 0.001	86.70%

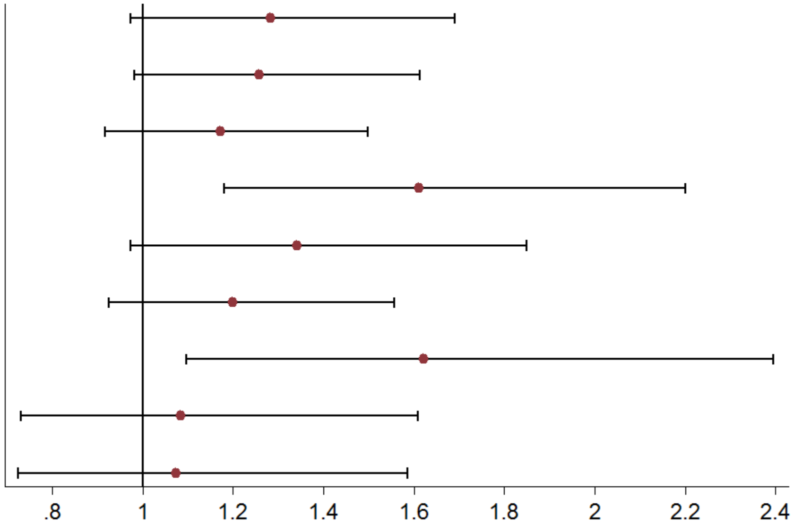


Fig 4.14 Sensitivity analysis for estimation of incidence rate ratio (IRR) of *ctpr7d* using all-predictor and univariable models.

Model	**P**	**IRR**	**95% CI**		**Heterogeneity P**	**I^2**
1. nb nk5 lag2 allvar	< 0.001	0.694	(0.566,	0.852)	0.038	45.30%
2. nb nk5 noAC allvar	0.001	0.688	(0.557,	0.850)	0.044	44.00%
3. nb nk5 logpre allvar	0.009	0.762	(0.621,	0.935)	0.040	45.00%
4. nb nk6 lag2 allvar	0.003	0.691	(0.540,	0.885)	0.009	57.30%
5. zinb nk5 lag2 allvar	0.003	0.731	(0.594,	0.899)	0.006	56.80%
6. zinb nk5 logpre allvar	0.025	0.807	(0.669,	0.974)	0.029	47.60%
7. zinb nk6 lag2 all var	0.017	0.747	(0.588,	0.948)	0.002	63.50%
8. nb nk5 lag2 univar	0.408	0.91	0.729	1.137	< 0.001	74.60%
9. zinb nk5 lag2 univar	0.561	0.939	0.759	1.161	< 0.001	78.10%

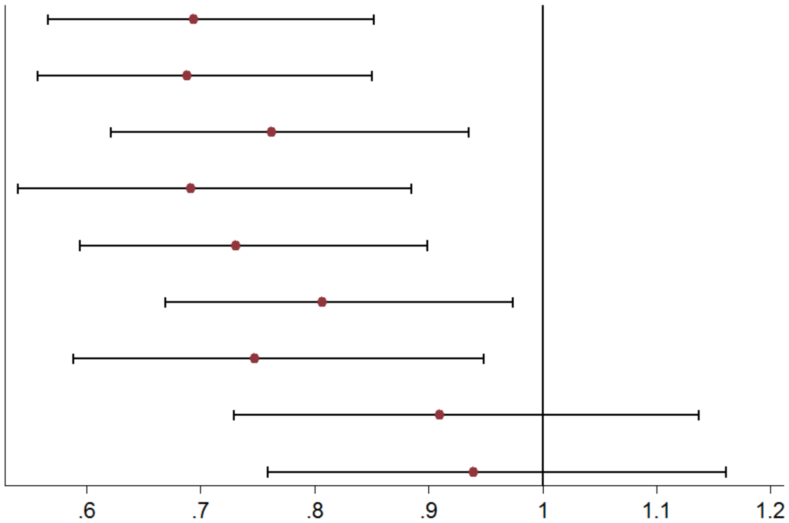


Fig 4.15 Sensitivity analysis for estimation of incidence rate ratio (IRR) of *wimp3d* using all-predictor and univariable models.

Model	**P**	**IRR**	**95% CI**		**Heterogeneity P**	**I^2**
1. nb nk5 lag2 allvar	0.058	1.213	(0.993,	1.481)	0.036	45.80%
2. nb nk5 noAC allvar	0.064	1.209	(0.989,	1.479)	0.062	40.80%
3. nb nk5 logpre allvar	0.078	1.137	(0.986,	1.311)	0.444	0.10%
4. nb nk6 lag2 allvar	0.145	1.192	(0.941,	1.509)	0.010	57.00%
5. zinb nk5 lag2 allvar	0.335	1.11	(0.898,	1.372)	0.001	63.30%
6. zinb nk5 logpre allvar	0.285	1.086	(0.933,	1.264)	0.16	28.30%
7. zinb nk6 lag2 all var	0.503	1.088	(0.849,	1.394)	< 0.001	70.60%
8. nb nk5 lag2 univar	0.730	1.031	0.867	1.226	0.006	56.70%
9. zinb nk5 lag2 univar	0.979	0.998	0.834	1.193	< 0.001	68.10%

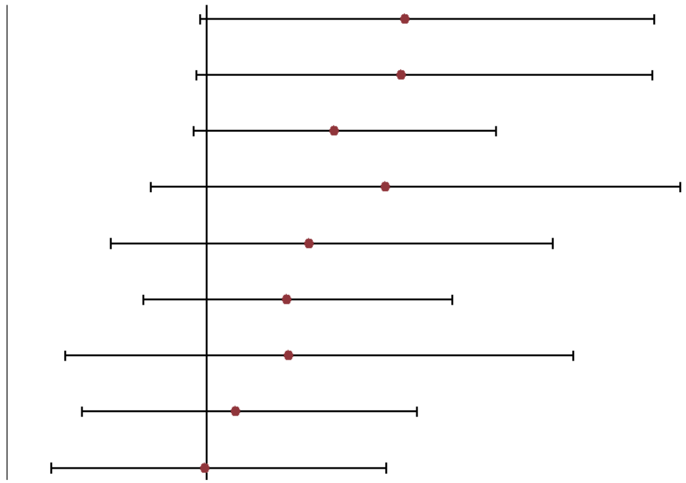
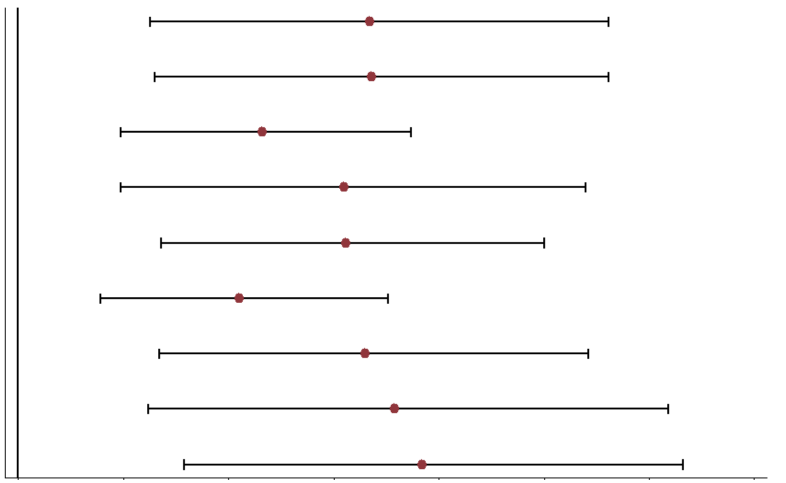
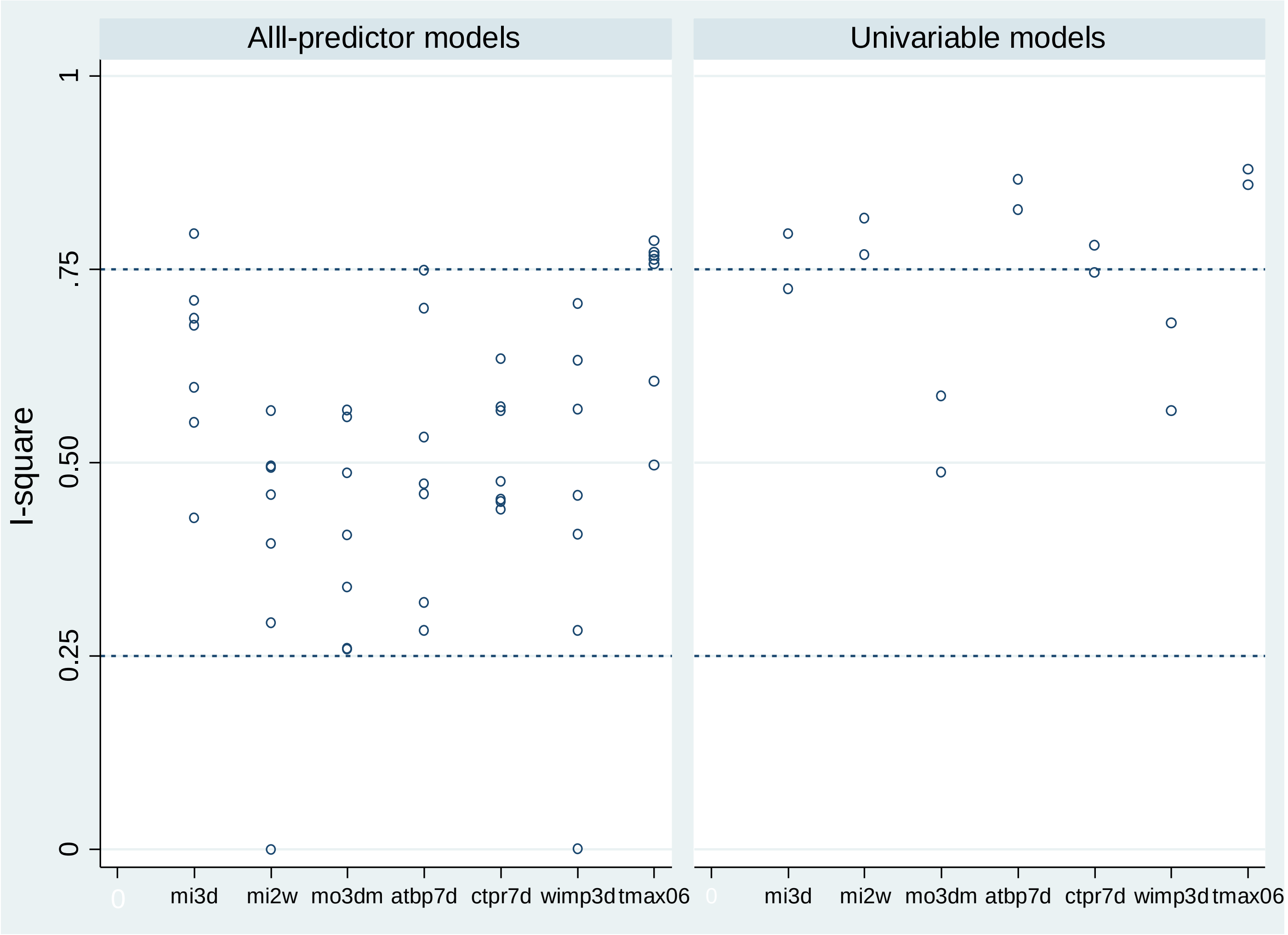


Fig 4.16 Sensitivity analysis for estimation of incidence rate ratio (IRR) of *tmax06* using all-predictor and univariable models.



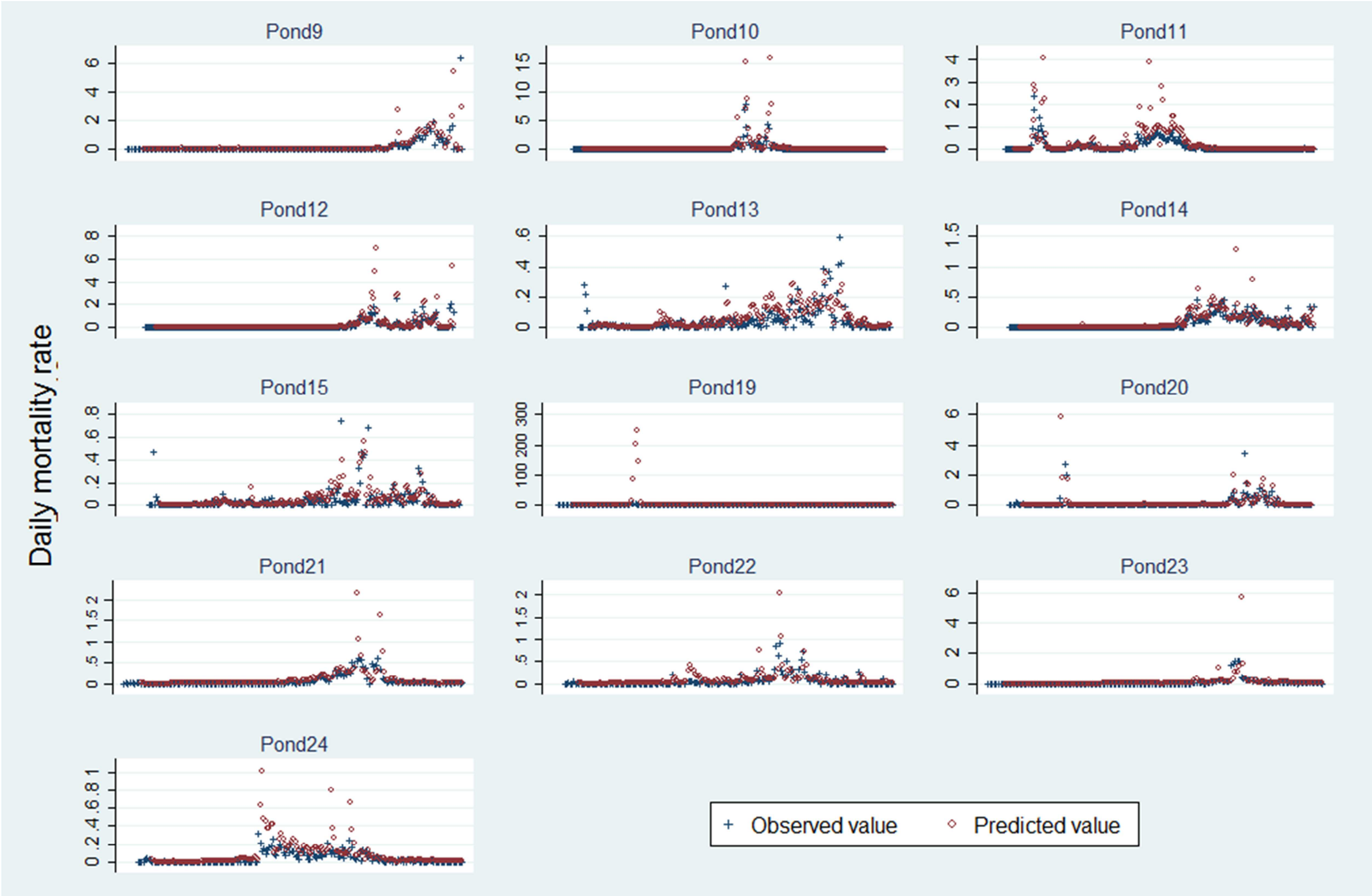
Model	P	IRR	95% CI	Heterogeneity P	I^2
1. nb nk5 lag2 allvar	0.001	1.167	(1.063, 1.281)	<0.001	78.70%
2. nb nk5 noAC allvar	0.001	1.168	(1.065, 1.281)	<0.001	76.30%
3. nb nk5 logpre allvar	< 0.001	1.116	(1.049, 1.187)	0.021	49.70%
4. nb nk6 lag2 allvar	0.003	1.155	(1.049, 1.270)	<0.001	75.70%
5. zinb nk5 lag2 allvar	< 0.001	1.156	(1.068, 1.250)	<0.001	76.80%
6. zinb nk5 logpre allvar	0.002	1.105	(1.039, 1.176)	0.002	60.60%
7. zinb nk6 lag2 all var	0.001	1.165	(1.067, 1.271)	<0.001	77.20%
8. nb nk5 lag2 univar	0.002	1.179	(1.062, 1.309)	<0.001	86.00%
9. zinb nk5 lag2 univar	0.001	1.192	(1.079, 1.316)	<0.001	88.00%

Fig 4.17 Values **of** I^{2*a} of the heterogeneity tests of the meta-analyses of all-predictor models (Settings of 1-7 listed in Table 4.5).



Note: *a The level of between-ponds inconsistency of coefficients estimated by the 7 models was assigned as low when $I^2 < 0.25$, moderate when $0.25 \leq I^2 < 0.75$, and high when $I^2 \geq 0.75$.

Fig 4.18 Observed and predicted values of daily mortality rate ($x10^3$)* by using main model for each pond



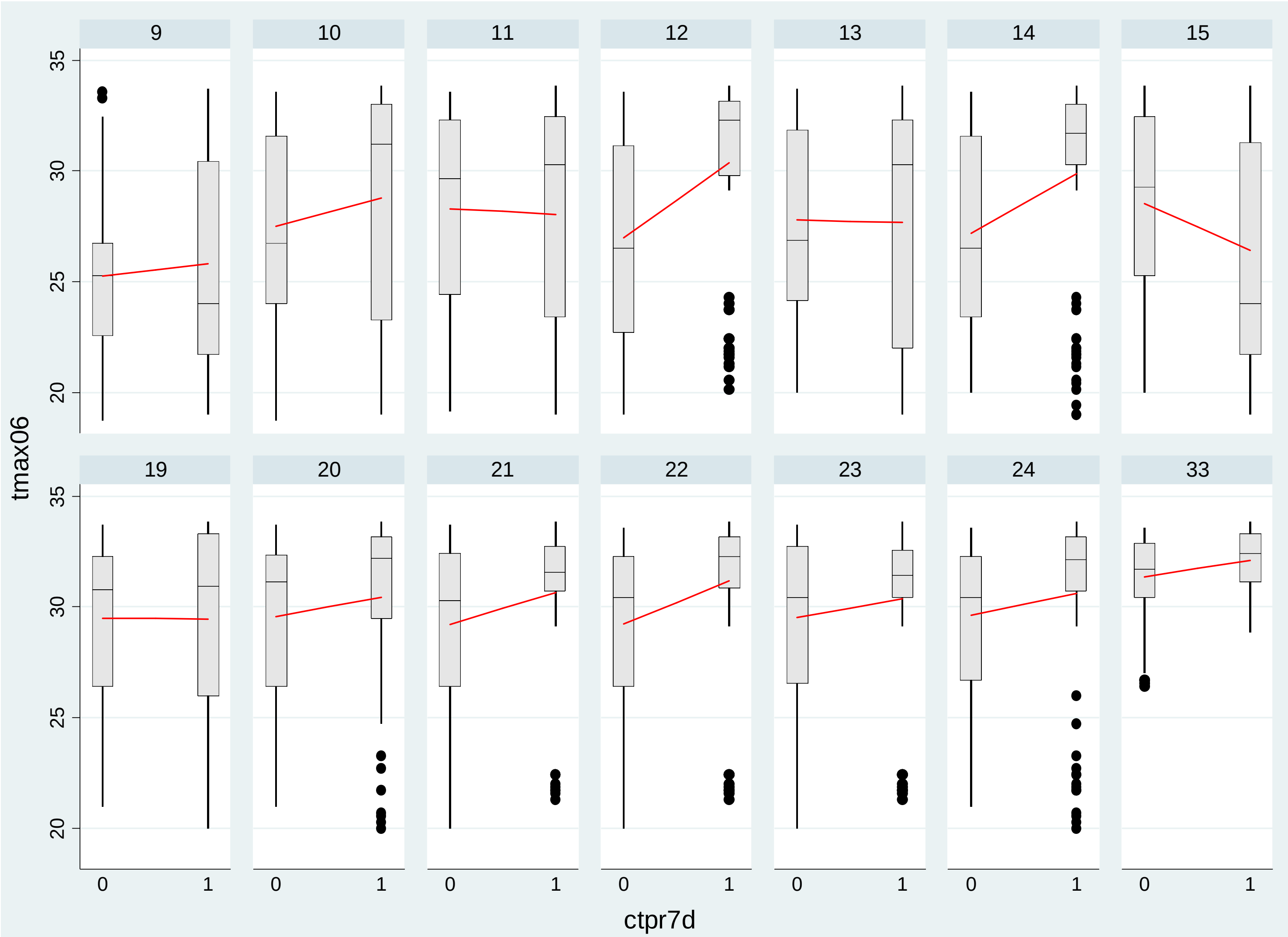
Note: * Daily mortality rates were defined as the ratio of the observed or predicted mortality counts divided by the total number of grass carp on the same day.

4.9. Supplementary materials for Chapter 5

Table S4.1 Estimated mean and 95% confidence intervals of each predictor calculated from the main model (indicated as the model of 2. nk5 lag2 in Table 4.5 for the data excluding ponds 19 and 23)

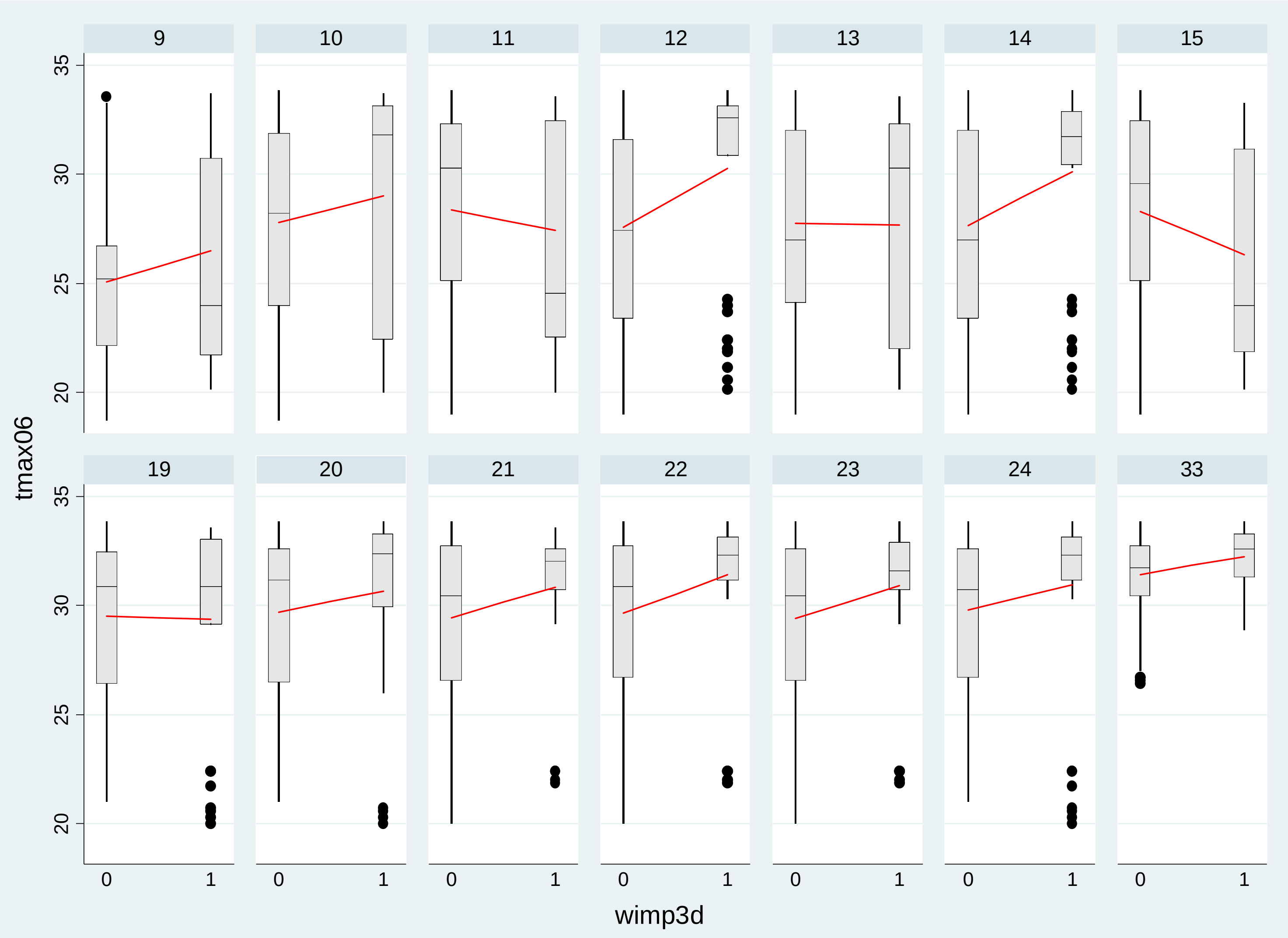
			95% Confidence interval	
Predictor variables and effects evaluated	**Coefficient**	**P**	**Lower**	**Upper**
Movementin of fish within previous 3 days (*mi3d*=1)	0.835	0.482	0.505	1.381
Movement in of fish within previous 14 days (*mi2w*=1)	1.893	<0.001	1.386	2.586
Movement out of fish within previous 3 days (*mo3d*=1)	1.581	0.052	0.997	2.508
Treatment of antibiotics or antiparasitics (*atbp7d*=1)	1.362	0.068	0.986	1.882
Treatment of TCM or antibiotics (*ctpr3d*=1)	0.688	0.002	0.546	0.867
Acute effect of water treatment (*wimp3d*=1)	1.201	0.099	0.966	1.494
Temperature of previous week increase by 1 (*tmax06*)	1.143	0.001	1.053	1.242

Fig S4.1 Pond-wise boxplots of *tmax06* of the scenario when *ctpr7d*=0 compared with that when *ctpr7d*=1.



Note: Red line is the connecting line of each group mean of the treatment group (ctpr7d=1) and non-treatment group (ctpr7d=0).

Fig S4.2 Pond-wise boxplots of tmax06 of the scenario when *wimp3d*=0 compared with that when *wimp3d* =1.



Note: Red line is the connecting line of each group mean of the treatment group (wimp3d=1) and non-treatment group (wimp3d =0).

Chapter 5 Supply chain management of live freshwater finfish in China: a case study of customer credibility evaluation using cross-classified modeling

5.1. Abstract

A case study was designed to investigate logistical factors influencing fish mortalities during transport, based on a company's concerns about undocumented mortality claims by customers. The data were the company's daily transaction records of the 3 species transported from Guangdong province to destination markets in Beijing between April and July 2013: largemouth bass *(Micropterus salmoides)*, Chinese perch (*Siniperca chuatsi)*, and longsnout catfish *(Leiocassis longirostris)*. Magnitudes and patterns of weekly mortalities of transported fish were quantified, and cross-classified random-effect modeling was used to explore variation and clustering of fish mortality claims at wholesale destinations. Random effects for customer, week, and market-week were interpreted by variance partition coefficients (VPC) and intraclass correlation coefficients (ICC). A significant fixed effect of market was found in the model of mortality claims for longsnout catfish ($p<0.05$), and changing patterns of VPC and ICC suggested that customers ordering longsnout catfish had more variation in claims than those ordering the other 2 species. Due to limited information on biological measurements in transported fish, the precise reason for these claims could not be determined. Some variations in mortality claims may be attributed to delivery factors. Future research should target areas such as fish physiological factors and transportation conditions, to better understand their role in reported mortalities. These findings indicated a need for better customer communication and improved technical parameters for live fish transportation.

Keywords: Live fish transportation, fish mortality claims, customer credibility, cross-classified model, China

5.2. Introduction

Freshwater finfish farmed in China are largely targeted for domestic markets (UN Comtrade 2013), where marketing of live fish is the most important form of retail. With the advantage of a climate that allows fish harvest all year round, most of the national freshwater fish transportation companies are located in the region of Pearl River Delta, in China. Farmed fish, especially high-value species, are transported on a daily basis from this region to wholesale markets in provincial capitals throughout the country. Live fish are seen as “value-added” products after their long-distance transportation to the markets (Chiu *et al.* 2013). The supply chain of live fish is, therefore, a critical component of the nation-wide provision of aquatic products to meet high market demands, and create profitability for local fish farmers.

However, information management is still a major bottleneck for the sustainability of the live fish supply chain. To our knowledge, no systematic studies have been carried out on the measurement of major problems in supply chain management (SCM) of live fish. Mortality of transported live fish is one of the most important concerns. Due to the lack of current techniques and facilities in live fish transportation, mortalities of transported fish have been reported at almost 1.5 million tons, annually, in China. Although large volumes of live fish are transported in China, no studies have been published to quantify fish mortality and factors associated with those mortalities.

Maintaining healthy live fish during prolonged transport can be problematic and is a key factor affecting the operational performance of the supply chain (De Silva, 2011). Health problems associated with transportation and post-delivery mortalities can severely limit the profitability of wholesale supplies (O’Keeffe, 2011; Grant et al., 2015). Physiological complications during transport, such as low oxygen levels, are believed to be responsible for most mortalities during transport (Hjeltnes et al., 2008; Wynne and Wurts, 2011).On the other hand, judgments made by wholesale customers about dead fish may involve some ambiguity about mortality measurements of fish at delivery (Fang and Tan, 2010; Wang, 2014). Credibility of customer complaints has become a key factor in the SCM and decision-supporting processes of live fish supplies in China (Stefanovic, 2014). It is important for SCM companies not only to determine the risks that exist along the supply chain, but also improvement of trust-based communications between the suppliers and customers can be meaningful to provide with a model for successful transport and compensation (Harper, 2010; Msimangira and Venkatraman, 2014). Furthermore, if there are no protocols for documenting mortality or morbidity during the delivery process, complications can arise when assessing the validity of customers' claims of mortality of purchased (or delivered) fish.

In this study, I explore how fish logistics companies can benefit from analyses of their transportation protocols and transaction records. According to anecdotal information from Company A, a leading live fish logistics company in China, Beijing markets receive the highest volume of fish among all of its transportation routes, with single-species orders of up to 5000 kg per day. However, mortalities claimed from Beijing markets are higher than the other transportation routes, which cause ongoing and serious financial losses to the company. A case study was designed to analyze the variation of fish mortality claims and identify potential factors affecting those claims. The specific purposes of the study were to assess whether there was clustering of fish mortality claims among customers over time, and to evaluate customer credibility.

5.3. Materials and methods

5.3.1. Data source

In Guangdong province, company X, one of the largest fish logistics companies in southern China, purchases farmed freshwater fish, and packages and transports about 40 thousand metric tons of live fish to more than 40 cities across the country.

(1) Fish sorting grading and holding. Immediately after fish are harvested at local farms, the company transports the fish in pond-water filled containers in trucks, outfitted with oxygen cylinders and gas diffusers. Within 2 to 4 hours of arrival at the company processing plant, fish are unloaded, scaled, sorted, and separated into storage tanks according to species and size. Two major treatments of fish occur during this holding period: body temperature is reduced, and fish are anesthetized with carbon dioxide (CO_2). Generally, fish are kept at 14-18 ^{0}C in holding tanks for 3-12 hours prior to packaging for live transport to wholesale markets.

(2) Fish packaging. Different species of live fish are separately packaged in styrofoam boxes with inside dimensions of 42cm x 28cm x 22cm (25.9 liter), containing water and crushed ice to maintain the water temperature between 10 and 12 ^{0}C, before the addition of fish. Each styrofoam box in the truck is sealed with tape and connected to an oxygen (O_2) cylinder via a plastic tube. A maximum of 600 boxes can be loaded on a single refrigerator truck. Market size (weight per fish) and packaging density (number of fish in each box) vary by species and season. The ranges of market sizes (kg per fish) of the 3 species involved in this study are as follows: 0.4-0.6 of largemouth bass, 0.75-1.5 of Chinese perch, and 0.75-1.0 of longsnout catfish. All 3 species are aggressive and carnivorous. After CO_2 anesthesia, approximately 10-25 kg of live fish can be packaged in a single styrofoam box.

(3) Live fish transportation. Two 30-ton refrigerated trucks transport live fish every day from Guangdong to the 5 Beijing markets, a distance of about 2300 km, which takes about 30 - 48 hours. One truck delivers to the biggest market (Market 1), and the other to Market 3, where some fish are off-loaded directly to customers at Market 3 and others are transferred to another truck and transported to Markets 2, 4, and 5. The time between truck arrival at market and when each customer receives fish is 1 - 3 hours. During transportation, oxygenation of the styrofoam containers is provided, and water is not usually exchanged until the final destination.

(4) Fish transactions. When trucks arrive at their respective destination markets, customers (wholesalers who order fish from the company and sell live fish in the markets) report to company staff the total fish weight and weight of dead fish in each box they receive. Staff report this information in the daily transaction record, and submit it to the company office in Guangdong. It takes about 2 - 4 hours to complete all transaction records for each day, with visits to the customers who purchased fish.

Among all destination wholesale markets in China, Beijing accounted for the largest volume of fish transportation routes for this company, and high fish mortality claims by customers in Beijing markets resulted in the highest financial losses for the company. I retrieved the company's daily transaction records for customers in 5 wholesale markets in Beijing, from mid-April to the end of July, 2013. Information in the daily records included customer identification (ID), market name (by location), dates of the corresponding transaction records, daily ordered weight of each fish species, and total daily received weight of live and dead fish.

There were 6 different types of species transported by this company but because of missing and limited data on minor species, 3 species were selected for evaluation in this study: largemouth bass (*Micropterus salmoides*), Chinese perch (*Siniperca chuatsi*), and longsnout catfish (*Leiocassis longirostris*). One customer was excluded in Market 5, which was the company's own store, from the analysis but I included them in the descriptive analysis as a separate customer for comparison.

5.3.2. Data preparation

The same procedures of data preparation and modeling were applied for each of the 3 species. All data processing was done in STATA13 (Stata Corp., College Station, TX, USA).

Aggregation of daily data to weekly data. In order to explore the variation of fish mortality claims at the week level, I aggregated the original daily data into weekly data for each customer. The aggregated dataset included the following variables: customer, market, week (transaction date), weekly-received live weight of each fish species, and weekly-received weight of dead fish. For each customer in each week, the total received weight in one week equaled the sum of the weight of live and dead fish.

Outcome variable and Box-Cox analysis. The outcome variable of interest was the weekly mortality claimed by each customer, calculated by dividing the total weekly dead weight by total weekly weight received. Violation of the model assumption was done with carrying out by Box-Cox analysis to determine the suitable transformation of the outcome variable (Box and Cox, 1964). For the sake of presentation, the same Box-cox scale, square-root transformation, was used for all three species.

Identification of market-week (delivery) as latent explanatory variable. The company delivered fish to customers in the same markets with the same trucks every day. Delivery information was not directly provided but embedded in the sale transaction records. This allowed to generate a new variable of weekly delivery by grouping week and market together. For each market, all the customers' mortality claims in a given one-week period shared the same value of this variable called market-week.

Consolidation of Markets 2, 4, and 5 into a single market. Deliveries to Markets 2, 4, and 5 were done in different trucks but at almost the same time daily. Considering the small sample size at these 3 markets and the potential shipment impact from the same truck from Guangdong to Beijing, I aggregated markets 2, 4, and 5 into a single market when modeling, and re-named this as Market 2. All descriptive analysis and modeling were based on the data for consolidated markets.

5.3.3. Conceptual introduction of statistical modeling

For each customer within a market, the claims across weeks constituted repeated measures. Additionally, customers' claims at a specific market in a given week shared the same source and market-week of fish, giving rise to another (and cross-classified) hierarchical structure, involving market-week combinations to represent the market-week (Fig 5.1). This data structure required a specific mixed-model with consideration of repeated measures, random effects, and cross-classification structure.

A cross-classified random-effect model (CCREM) was constructed for the weekly mortality claimed by each customer to explore the variance structures and determine how those factors (market, market-week, and customer) were potentially associated with variation of high mortality claims. Market was regarded as a fixed effect, and random-effect terms included customer and market-week. It was assumed that normal distributions for random effects and the error term were normally distributed and had equal variance (Equation 5.1).

$sqrt\,(mort_{ijk}) = \mu + \alpha_i + u_j + v_{ik} + \varepsilon_{ijk}$ (Equation 5.1)

where, i ~ market, j ~ customer, *and* k ~ week, and

$$u_j \sim N(0, \sigma^2_{customer})$$
$$v_{ik} \sim N(0, \sigma^2_{market-week})$$
$$\varepsilon_{ijk} \sim N(0, \sigma^2_k)$$

For the between-week errors $(\varepsilon_{ijk})_k$, different covariance structures were explored: compound symmetry, first-order autoregressive, first-order autoregressive moving average, Toeplitz, as well as heterogeneous autoregressive and heterogeneous Toeplitz, of which the last two allowed for unequal variance (σ_k^2) across weeks.

5.3.4. Data analysis: descriptive analysis and CCREM modeling

Weekly mortalities reported were summarized for each customer and for each market. After testing overall market effects by a multiple Wald test, pairwise comparisons were also done to compare means between markets.

CCREM modeling was performed separately for each species using PROC MIXED in SAS 9.1.2 (SAS Institute Inc., Cary, NC, USA). In general, the analysis followed the principles described in Pineheira & Bakes (2000). Maximum likelihood estimation was used, and the best-fitting covariance structure was determined by Akaike's information criterion (AIC).

In order to facilitate interpretation of variance parameters, partition variance components for the purpose of trend detection and estimation, 2 types of coefficient were calculated to examine how mortality variation could be attributable to customers, shipment, or other unexplained factors: variance partition coefficients **(**VPC) and intraclass correlation coefficients **(**ICC) were used. VPC reflects the variation at different levels of the hierarchy, and ICC indicates the homogeneity of observations sharing the same units of hierarchical structure (Goldstein, 2002; Dohoo et al., 2009).

(1) Variance partition coefficient. The VPC expressed the percentage of variance across customers out of the total variance (Equation 4.2). The unexplained variance during specific weeks (σ_k^2), potentially related to factors not included in the model, i.e. transportation conditions (e.g. driver, packaging conditions).

$$VPC(week_k) = \frac{\sigma^2_{customer}}{\sigma^2_{customer}+\sigma^2_{market-week}+\sigma^2_k} \quad \text{(Equation 5.2)}$$

where,

$\sigma^2_{customer}$, overall variance among customers

$\sigma^2_{market-week}$,variance attributed to the market deliveries during a specific week

σ^2_k , unexplained variance among customers at specific week

(2) Intraclass correlation coefficient represents the percentage of variance explained by the customer when the market effect is removed from the total variance (Equation 5.3).

$$ICC(week_k) = \frac{\sigma^2_{customer}}{\sigma^2_{customer}+\sigma^2_k} \quad \text{(Equation 5.3)}$$

In order to identify customers with extremely high claims of fish mortalities, I computed best linear unbiased predictors (BLUP), and ranked customers based on these BLUP estimates.

Diagnostics in the mixed model were based on BLUPs and Studentized marginal residuals, using plots of residuals versus predicted values and Q-Q plots, and identifying the highest and lowest residual values. Square-root transformation of the weekly mortality claims ensured satisfactory model diagnosis.

5.4. Results

5.4.1. Descriptive analysis

5.4.1.1. Unbalanced data structure

Total 8094 daily records from 3 markets were aggregated to generate 415, 436, and 283 weekly data points for the 3 species (Table 5.1). The unbalanced structure existed because different numbers of customers were distributed within each market, the numbers of orders were different among markets and among customers across weeks.

The total transportation of live fish weight also varied across weeks. Mean daily orders for different weeks, pooling all markets, ranged from 177 kg to 4700 kg, 424 kg to 3097 kg, and 509 kg to 1836kg for largemouth bass, Chinese perch and longsnout catfish, respectively. There was an apparent decrease in orders of largemouth bass in the first week of July 2013.

5.4.1.2. Weekly biomass claimed as mortality across customers and weeks

Customers were found to claim differently for mortality in the 3 species (Figs 5.2, 5.3a and 5.3b). Patterns in mortality suggested some weeks were worse than others for claims and had more variation in claims and these patterns differed among the 3 species (Fig 5.2). The species with the overall highest number of claims was Chinese perch (Figs 5.3a and 5.3b).

Among customers, the single case of highest weekly mortality reported from Market 1 was 69% for largemouth bass, 100% for Chinese perch, and 64.5 % for longsnout catfish. The highest individual customer claims for the 3 species occurred in weeks of 27, 31, and 26 respectively, for largemouth bass, Chinese perch and longsnout catfish. The highest means of weekly claims across all customers occurred during weeks 27, 21, and 28, respectively, for largemouth bass (29.6%), Chinese perch (49.7%) and longsnout catfish (34.6%), in which the Chinese perch had the highest average weekly mortality claims. The species with the lowest average mortality claims was largemouth bass (16.1%). The highest weekly variance of mortality claims, based on the square-root scale, occurred in weeks 27, 18 and 15, respectively, for largemouth bass (0.07), Chinese perch (0.05) and longsnout catfish (0.05).

5.4.2. Cross-classified random-effect modeling

5.4.2.1. Unbalanced Model selection

Based on the AIC model selection criterion, the same heterogeneous autoregressive structure was chosen for all species in order to facilitate the interpretation of our modeling results across the 3 species.

5.4.2.2. CCREM using candidate models

(I) Estimation of fixed effects

The market effect was only significant for longsnout catfish (Table 5. 2). Markets 2 and 3 were significantly different than Market 1, but were not different from each other (Table 5.2).

(II) Estimation of random effects

Overall customers and market-week. Similar random effects of customer and market-week were found for largemouth bass and Chinese perch (Table 5. 2). However, for longsnout catfish, customer's random effect was much greater (Table 5. 2).

Week variation. There was variation across weeks in the unexplained variance for the different species (Table 5.2), ranging from 0.004-0.066 for largemouth bass, 0.005-0.039 for Chinese perch, and 0.002-0.031 for longsnout catfish.

There was only moderate auto-correlation among weeks between fish mortality claims, and it was slightly stronger for Chinese perch than other species (Table 5.2).

Variance partition coefficients and intraclass correlation coefficients. VPC and ICC estimates calculated from the longsnout catfish model were generally higher than those from the largemouth bass and Chinese perch models. For example, the highest VPC for each species was 0.206 for largemouth bass, 0.198 for Chinese perch, and 0.607 for longsnout catfish. The VPC calculated per week in the longsnout catfish model was always higher than 0.315 (Table 5.2), showing a consistently high variation between customers.

(III) Prediction of random effects

The BLUPs plots showed a stronger variability among predicted customer random effects for customers ordering longsnout catfish compared with the other 2 species (Figs. 5.4-6). Customers 20, 45, and 44 from Market 2 appeared in the top 10 BLUP values from models of largemouth bass and Chinese perch (Figs. 5.4-5). Customer 20 was listed in the top ranks across all 3 species. Customers with higher rankings in the longsnout catfish were mostly from Market 1 (Fig 5.6). Of the 12 customers with the highest predicted estimates, all but 2 came from Market 1. There were 4 other customers with low predicted estimates.

The models for the 3 species largely met the normality assumption, except for a few outliers. Sensitivity analyses were done respectively for removal of outliers and ideal scale of Box-Cox transformation for each species, and the results confirmed that both outliers and Box-Cox transformation had minimal impact on general conclusions and other detailed model results (Tables S5.1-2).

5.5. Discussion

Variation of mortality claims were found for all 3 species across markets, and patterns of mortality claims varied by customer, market-week (delivery), and week. In addition, significant among-market differences were found for longsnout catfish.

The highest claims for this species were from customers in Market 1. The high VPC of longsnout catfish during most weeks indicated that customer consistently explained most of the variation. In other words, the unexplained variation was low relative to among-customer variation. This high customer effect was mainly attributable to a few customers always claiming higher mortalities. The weeks with low VPC for this species suggested that there was relatively more unexplained variance, indicating that some customers comparatively claim quite differently to how they generally do. Higher variation of claims of longsnout catfish occurred in some weeks when mortalities of 1 or 2 deliveries were extremely high compared to others deliveries in the same weeks. This could have occurred if the breakdown of packaging boxes in the truck occurred or transport was delayed for other less-controlled events, such as during unexpected weather conditions.

For largemouth bass, customers inconsistently claimed high or low mortalities, suggesting customers only explained minor variations in mortality claims. Compared with the other 2 species, this species had lower mortality claims, possibly because its market price (estimated USD 3.87/ kg) was much lower than for the other 2 species (Chinese perch was USD 9.68/kg, and largemouth catfish was USD 4.52/kg). As well, this species likely survives transport better than other 2 species because of its smaller market size and higher tolerance of low O_2 and high ammonia. Weekly variation in this species indicated that either some customers over- or under-claimed mortality, or some customers frequently claimed fish mortality differently, but high or low claims were not from the same individual customers (i.e. overall low customer effect).

For this highest-claim species, Chinese perch, there were several high ICC weeks when most of the customers claimed high mortalities across the markets, which might be related to both delivery and fish biology factors, i.e. packaging density, water quality, and drivers' attention to oxygen meters during transport. There were also a few low ICC weeks that also coincided with high mortality. During these weeks, it is possible that the majority of the claims were made by only a few customers, but this tended to be the exception.

Compared to the other 2 fish species, Chinese perch is particularly prone to transport mortality because they are shipped at a larger market size, they are more prone to O_2 problems, are more expensive than the other species, and less tolerant to high ammonia and low O_2. Although I were unable to assess whether mortality clustered at the level of deliveries or containers, the fact that so many customers were claiming on the same weeks suggests mortality claims of Chinese perch were likely valid. In addition, the value of Chinese perch apparently decreases when loss of pigmentation occurs after transport. Customers more likely claimed mortality based on pigmentation reduction if the fish had been transported long distances.

Since no fish-logistics company can ensure that all fish are delivered to market without any mortality, customer communication is important in supply chain management. I examined whether specific customers purchasing multiple species complained more than others. Of the 10 customers with the highest predicted parameter estimates for Chinese perch, 4 were also on the top-10 list for the other 2 species, and 6 were on the top-10 list for largemouth bass, suggesting that these 10 customers may complain more than others. However, given our findings, it is likely that mortality in fish transports occurs commonly among species and fish that are more prone to poor water quality were more affected, which suggests customers are not falsely claiming mortality at, at least, weeks where the VPC values are not high. It was interesting to note that the tertiary markets did not have higher claims than Market 1, which was the first delivery point. I had expected worse results in the tertiary markets because of no water change until the unloading of fish at destinations.

Another question: were specific weeks worse for claims than others? The summer weeks, defined as the period between June 2 and the end of July, had the higher mortality claims across all species. Higher claims were evident in all 3 species in week 27 (Fig 5.3). Interestingly, a few high-mortality weeks for specific species were those that had the smaller deliveries, i.e. week 27. This observation was also confirmed by the company which indicated that there were fewer claims when demand was high than in weeks with low demand. This might explain why the company was skeptical of some of the claims, as it may reflect market trends and not true mortality. However, during week 27, only some deliveries had high mortality claims and these were consistent across markets and all customers (Figs 5.2, 5.3a, and 5.3b) suggesting “real” mortality problems with these deliveries. To rule out the potential negative impact from deliveries with extreme-mortality, there were no deliveries where the fish mortalities were claimed 100% by all customers.

Causes of mortality during fish transport might be attributable to stress, water quality deterioration because of low O_2 concentrations, and buildup of metabolic waste and ammonia (Ashley 2007), given transport times of more than 30-40 hours. Stress in fish can occur throughout the whole process of harvesting, handling, packaging, loading, and transport until unloading at destination markets (Conte, 2004; Portz et al., 2006; Hjeltnes et al., 2008; Sung et al., 2011). Long periods of overcast days might have negatively impacted fish health before transportation (Mosig John and Fallu 2012; Thomas Lawson 2013). According to the company’s anecdotal notes, high mortality was often found more in female fish transported during the spawning season than in non-spawning season.

For closed transport with high fish density and low water flow, critical water quality parameters (e.g. temperature, dissolved oxygen, total ammonia nitrogen, CO_2) and foam formed by dissolved organic compounds) could become physiological stressors (Hjeltnes *et al.* 2008). Furthermore, when hundreds of boxes are stacked together, some oxygen tubes could be compressed, which might lead to extremely high mortality. Strategies to reduce transport mortalities might include starvation before harvest to reduce fecal loading in the transport containers (Lim et al., 2003), sedation in the holding tank to reduce oxygen demand (Bernier and Randall, 1998), acclimation of fish to ambient water temperate, pH and salinity control by reducing water temperature, and adding buffers and salts to packaging water (HSA, 2006; Wynne and Wurts, 2011).

Company X has 20-years' experience in live fish transportation; however, water quality management is still an ongoing challenge. Our study indicated that the company should identify critical control points, and we suggest the following defect action points (DAPs) (Lauzon, 2010; Wynne and Wurts, 2011; Codex Committee on Fish and Fishery Products, 2012): 1) health status of fish at the source; 2) harvest procedures and stress reduction during harvest; 3) handling of fish during sorting and packaging, i.e., fish size, and quality of styrofoam boxes; and 4) transportation preparation and timely monitoring, including preparation of truck cooling system, oxygen tubes, and water quality measurement (Singh, Burgess and Singh 2008; Froese 1998).

5.6. Conclusions

In conclusion, the analysis in this study suggests that customers are not the only factor responsible for variation in fish mortality claims, though some customers might explain claim variation more in longsnout catfish than in the other 2 species. Variation in claims of Chinese perch was more likely to be actual mortality and strongly associated with market-week, potentially related to that species' susceptibility to poor water quality and its higher market price. To my knowledge, this study is the first in warm water aquatic animals to use cross-classified modeling to partition the variability in mortality, specifically mortality claims, across different factors in the transportation chain. Further studies should evaluate reasons for fish mortalities, specifically at the individual delivery level, in order to improve fish health at market delivery.

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Table 5.1 Number of weekly transaction records of the 3 species in each destination market in Beijing during the 16-week time frame (weeks of 15-31 with week 17 not included).

Markets	**Largemouth bass** (*Micropterus salmoides*)	**Chinese perch** (*Siniperca chuatsi*)	**Longsnout catfish** (*Leiocassis longirostris*)
1	239	248	156
2	76	80	59
3	100	108	68
Overall	415	436	283

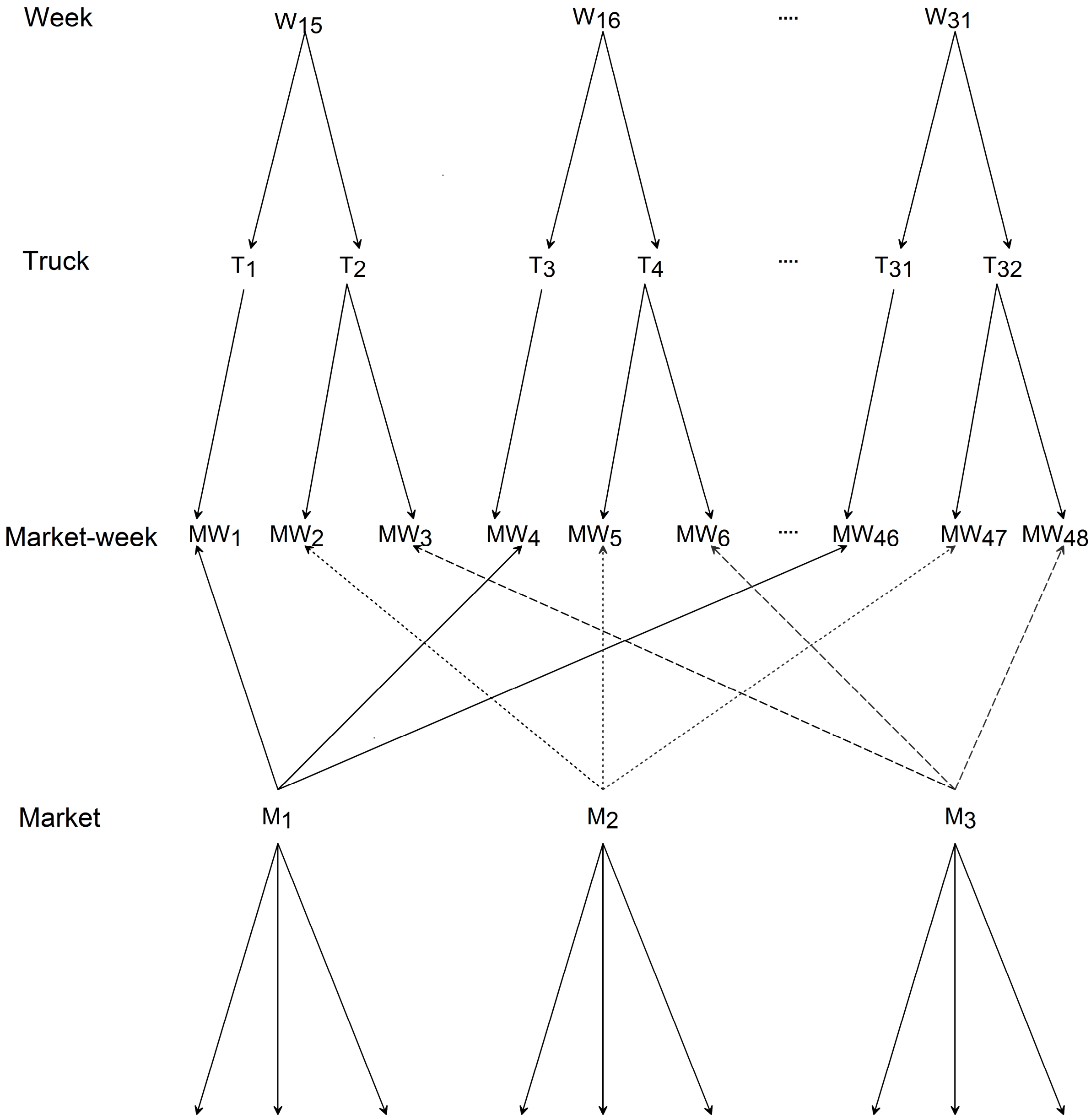
Months	**Week**	**First day of week**	**Number of weekly transactions aggregated**
April	15	4/7/2013	79
	16	4/14/2013	75
	18	4/28/2013	74
May	19	5/5/2013	73
	20	5/12/2013	74
	21	5/19/2013	75
	22	5/26/2013	72
June	23	6/2/2013	70
	24	6/9/2013	70
	25	6/16/2013	72
	26	6/23/2013	76
July	27	6/30/2013	67
	28	7/7/2013	60
	29	7/14/2013	64
	30	7/21/2013	66
	31	7/28/2013	67

Table 5.2 Fixed effect and random effects estimated by modeling with heterogenous autoregression covariance structure for data of the 3 fish species

	Largemouth bass (*Micropterus salmoides*)				**Chinese perch** (*Siniperca chuatsi*)				**Longsnout catfish** (*Leiocassis longirostris*)			
Fixed effect	**Estimate**	**95% CI*[a]**			**Estimate**	**95% CI**			**Estimate**	**95% CI**		
Intercept	0.341	0.278 - 0.404			0.505	0.438 - 0.572			0.498	0.406 - 0.590		
market2	0.040	-0.058 - 0.138			0.023	-0.081 - 0.127			-0.222	-0.370 - -0.069		
market3	0.024	-0.074 - 0.122			-0.017	-0.119 - 0.085			-0.273	-0.430 - -0.118		
Random effects	**Estimate**	**SE**	**VPC*[b]**	**ICC*[c]**	**Estimate**	**SE**	**VPC**	**ICC**	**Estimate**	**SE**	**VPC**	**ICC**
$\sigma^2_{customer}$	0.004	0.002			0.004	0.002			0.019	0.007		
$\sigma^2_{market-week}$	0.011	0.003			0.013	0.003			0.010	0.003		
Week (15) σ^2_{k15}	0.020	0.006	0.117	0.168	0.026	0.008	0.084	0.121	0.029	0.011	0.333	0.400
Week (16) σ^2_{k16}	0.004	0.001	0.218	0.503	0.006	0.002	0.157	0.363	0.014	0.006	0.448	0.579
Week (18) σ^2_{k18}	0.007	0.002	0.189	0.374	0.030	0.009	0.076	0.106	0.006	0.002	0.557	0.776
Week (19) σ^2_{k19}	0.011	0.003	0.162	0.281	0.013	0.004	0.120	0.213	0.008	0.003	0.528	0.721
Week (20) σ^2_{k20}	0.011	0.003	0.161	0.278	0.007	0.003	0.154	0.346	0.004	0.002	0.590	0.843
Week (21) σ^2_{k21}	0.007	0.003	0.189	0.373	0.005	0.002	0.164	0.404	0.013	0.005	0.465	0.608
Week (22) σ^2_{k22}	0.010	0.003	0.168	0.298	0.016	0.006	0.110	0.182	0.008	0.003	0.516	0.698
Week (23) σ^2_{k23}	0.006	0.002	0.194	0.392	0.003	0.001	0.182	0.532	0.007	0.003	0.538	0.740
Week (24) σ^2_{k24}	0.007	0.002	0.186	0.361	0.011	0.003	0.132	0.254	0.002	0.001	0.615	0.893
Week (25) σ^2_{k25}	0.014	0.004	0.143	0.228	0.015	0.005	0.113	0.191	0.024	0.009	0.362	0.443
Week (26) σ^2_{k26}	0.021	0.006	0.114	0.163	0.009	0.003	0.143	0.295	0.010	0.004	0.496	0.663
Week (27) σ^2_{k27}	0.066	0.022	0.051	0.059	0.035	0.010	0.070	0.094	0.012	0.005	0.468	0.614
Week (28) σ^2_{k28}	0.008	0.003	0.180	0.340	0.004	0.002	0.179	0.506	0.015	0.007	0.437	0.561
Week (29) σ^2_{k29}	0.012	0.004	0.153	0.256	0.010	0.004	0.133	0.257	0.007	0.003	0.539	0.742
Week (30) σ^2_{k30}	0.035	0.011	0.082	0.105	0.021	0.006	0.097	0.149	0.009	0.004	0.505	0.679
Week (31) σ^2_{k31}	0.004	0.002	0.213	0.482	0.039	0.012	0.064	0.084	0.031	0.013	0.320	0.383
autocorrelation	0.192	0.068			0.338	0.131			0.245	0.094		

Note: *[a] Confidence interval *[b]. VPC, Variance partition coefficient; *[c]. ICC, Intraclass correlation coefficient.

Fig 5.1 Data structure of week, market-week (delivery), market, customer, for largemouth bass[a]* between mid April (week 15) and the end of July (week 31).



Note: [a] All 3 species have the same data structure, and largemouth bass is used here for illustration purposes.

[b] C_1 here denotes the first customer sequentially counted in the markets, but not the customer ID, i.e., 17 customers in Market 1 has ordered largemouth bass during the study period.

Fig 5.2 Patterns of weekly mortality claimed by customers for 3 fish species between mid April (week 15) and the end of July (week 31).

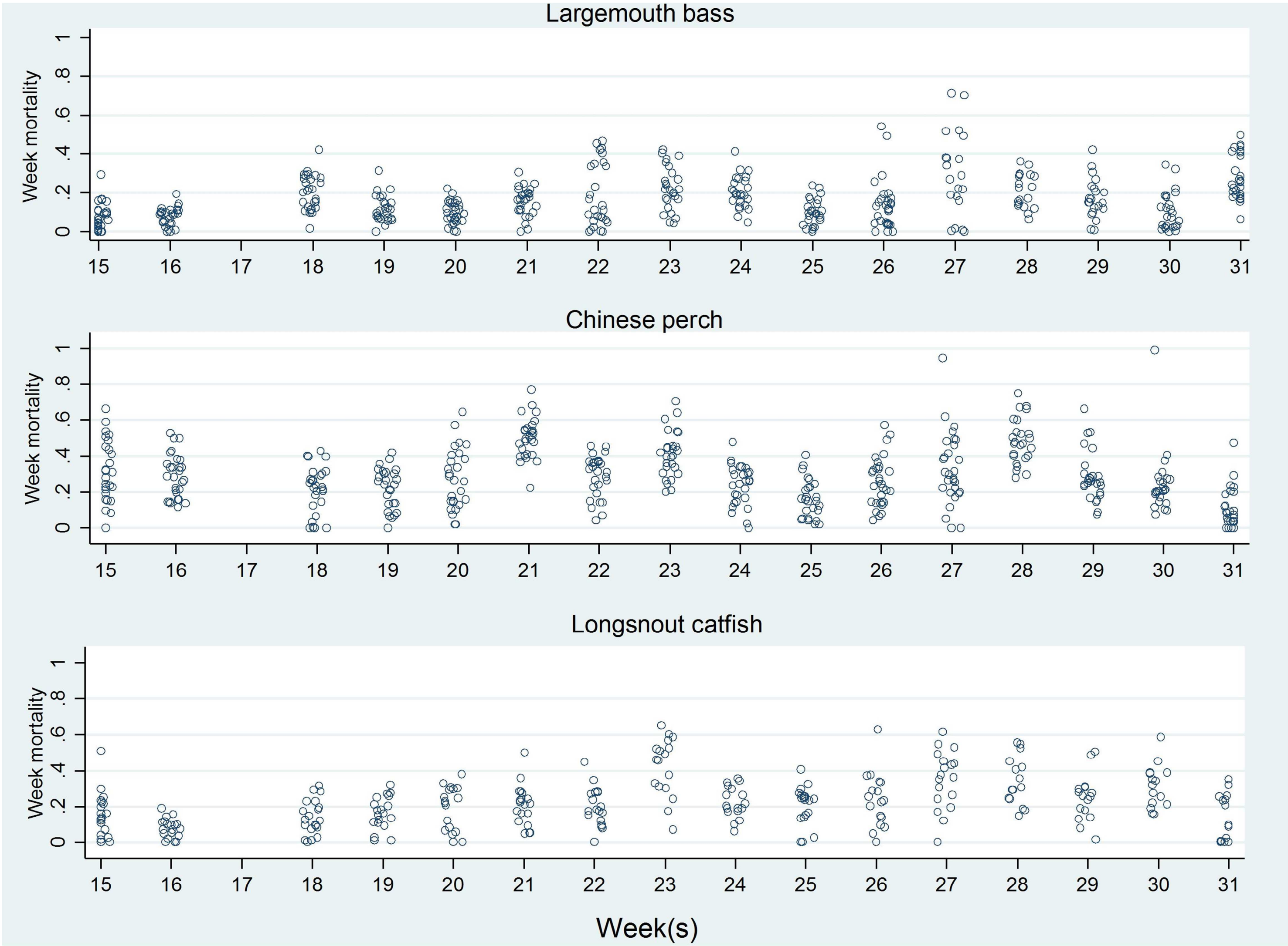
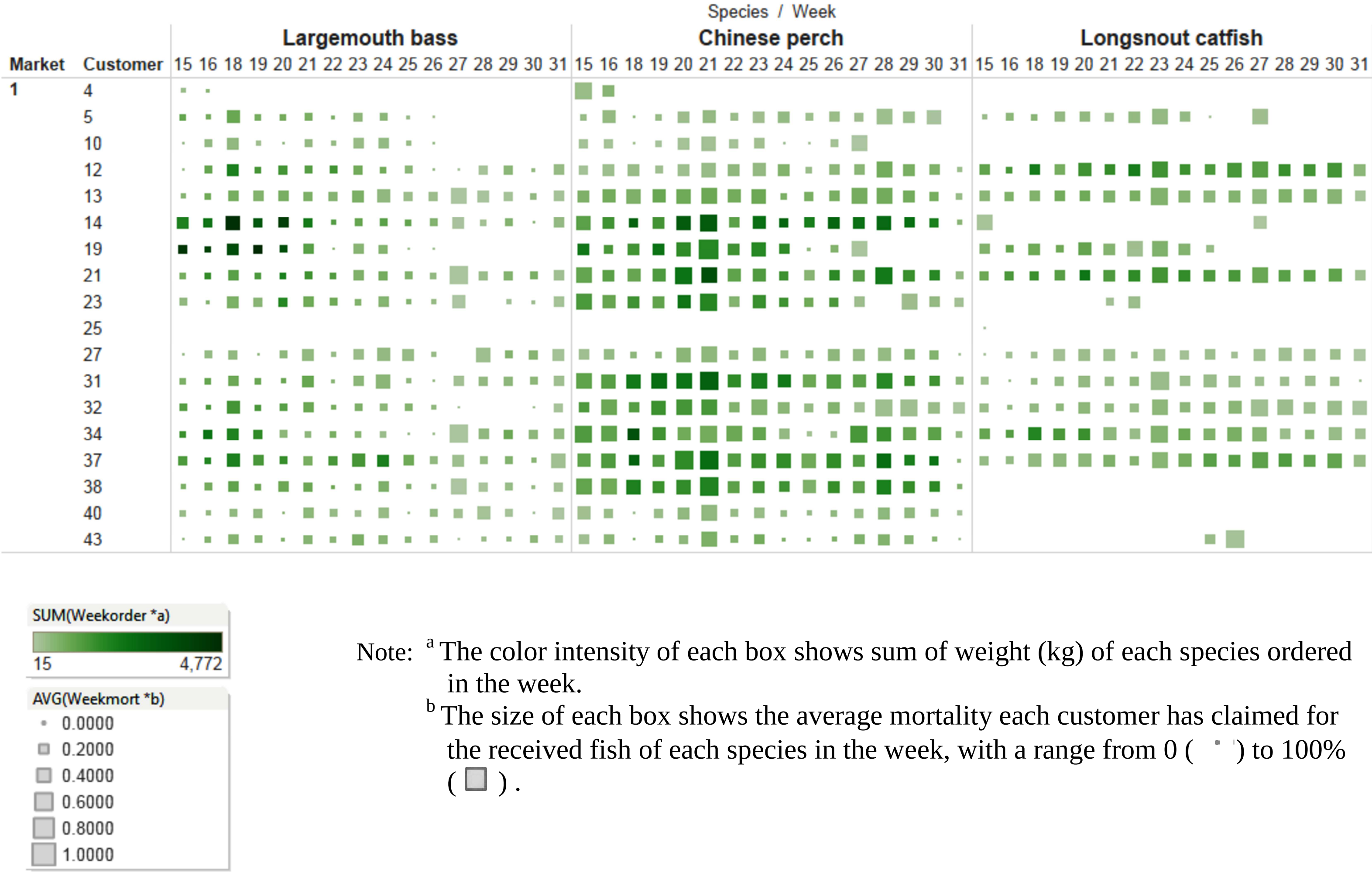
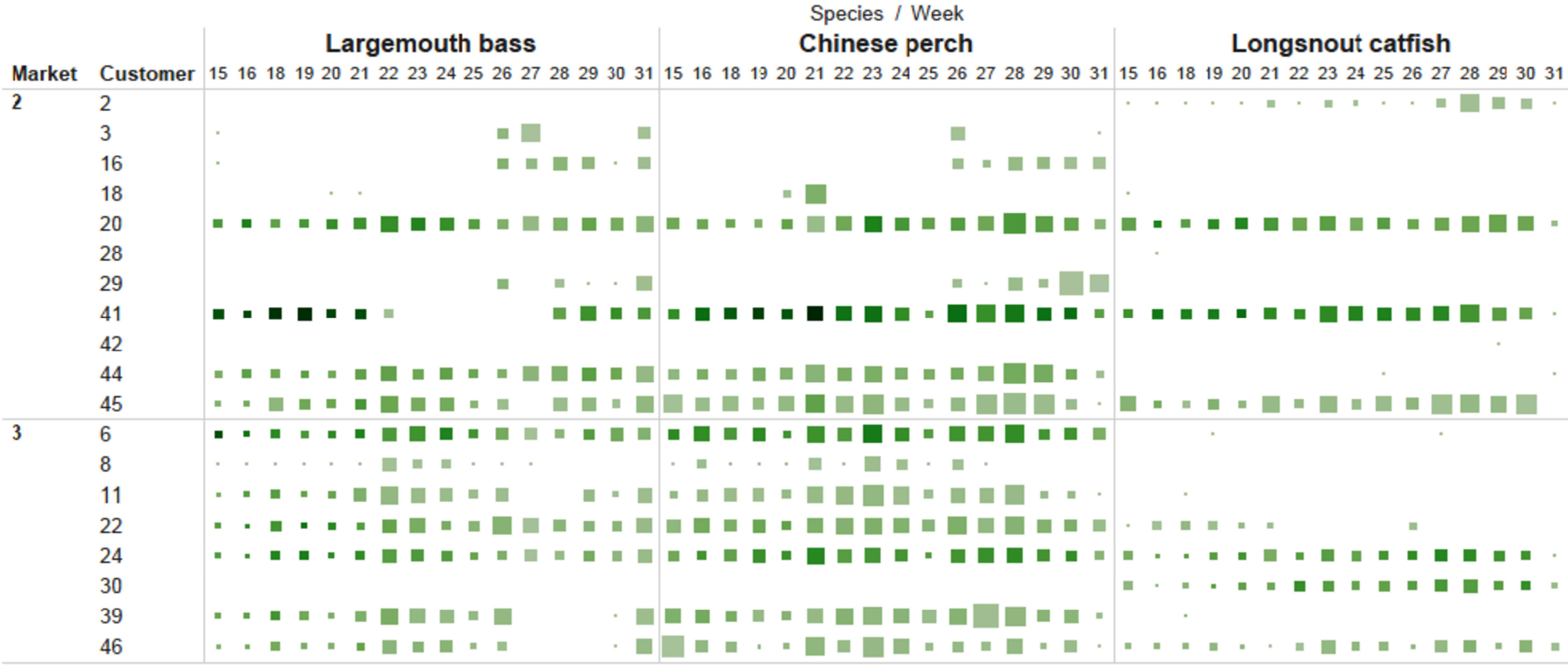


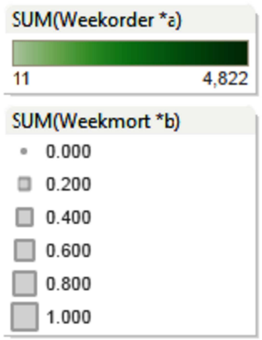
Fig 5.3a Weekly orders of each customer and weekly mortality of the 3 species claimed by customers in Market 1.



Note: [a] The color intensity of each box shows sum of weight (kg) of each species ordered in the week.
[b] The size of each box shows the average mortality each customer has claimed for the received fish of each species in the week, with a range from 0 (▪) to 100% (☐) .

Fig 5.4b Weekly orders of each customer and weekly mortality of the 3 species claimed by customers in Markets 2 &3.





Note: [a] The color intensity of each box shows sum of weight (kg) of each species ordered in the week.
[b] The size of each box shows the average mortality each customer has claimed for the received fish of each species in the week, with a range from 0 (·) to 100% (□) .

Fig 5.5 Ranking of customers ordering largemouth bass by best linear unbiased predictor (BLUP) estimates (with ± SE).

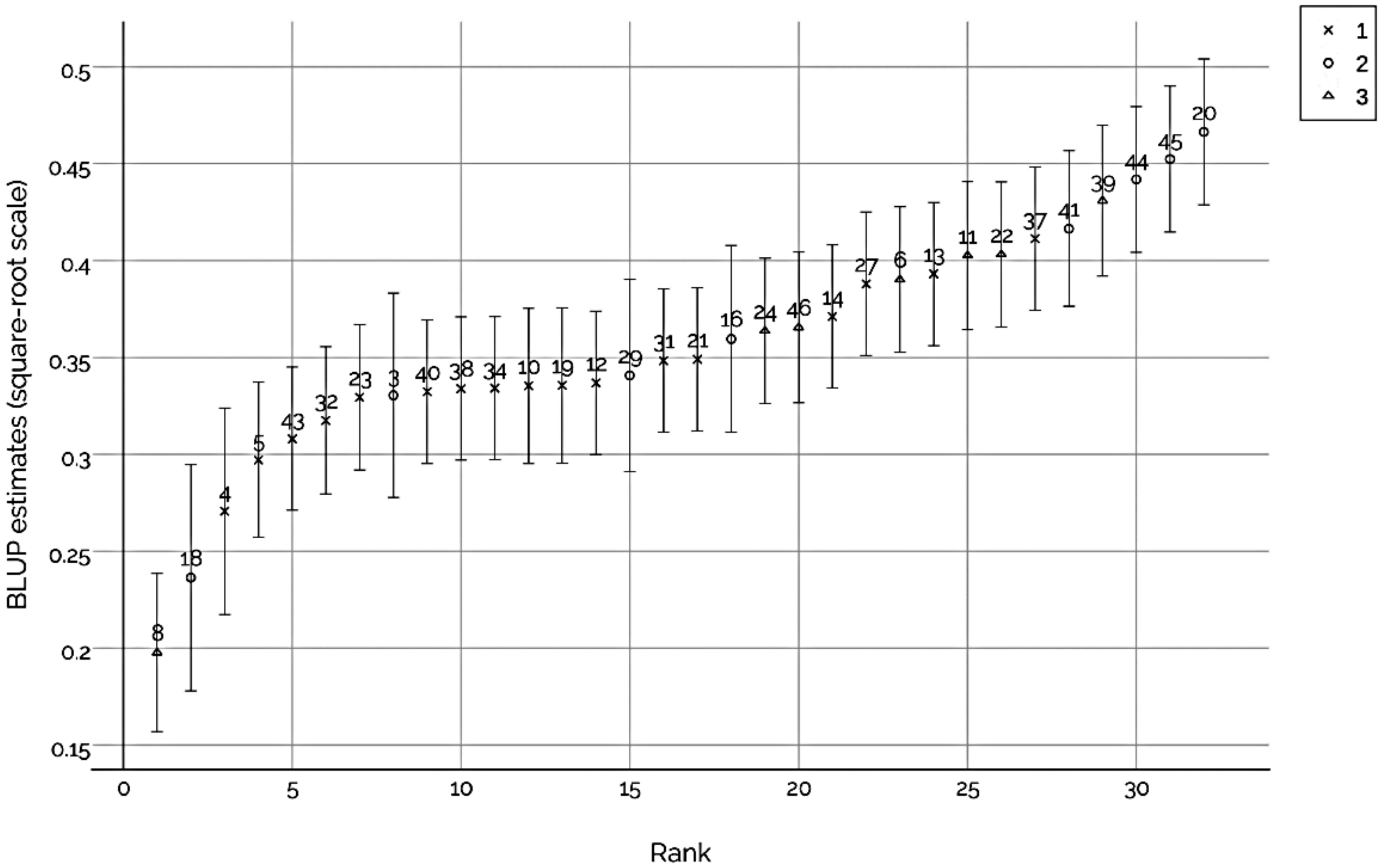


Fig 5.6 Ranking of customers ordering Chinese perch by best linear unbiased predictor (BLUP) estimates (with ± SE).

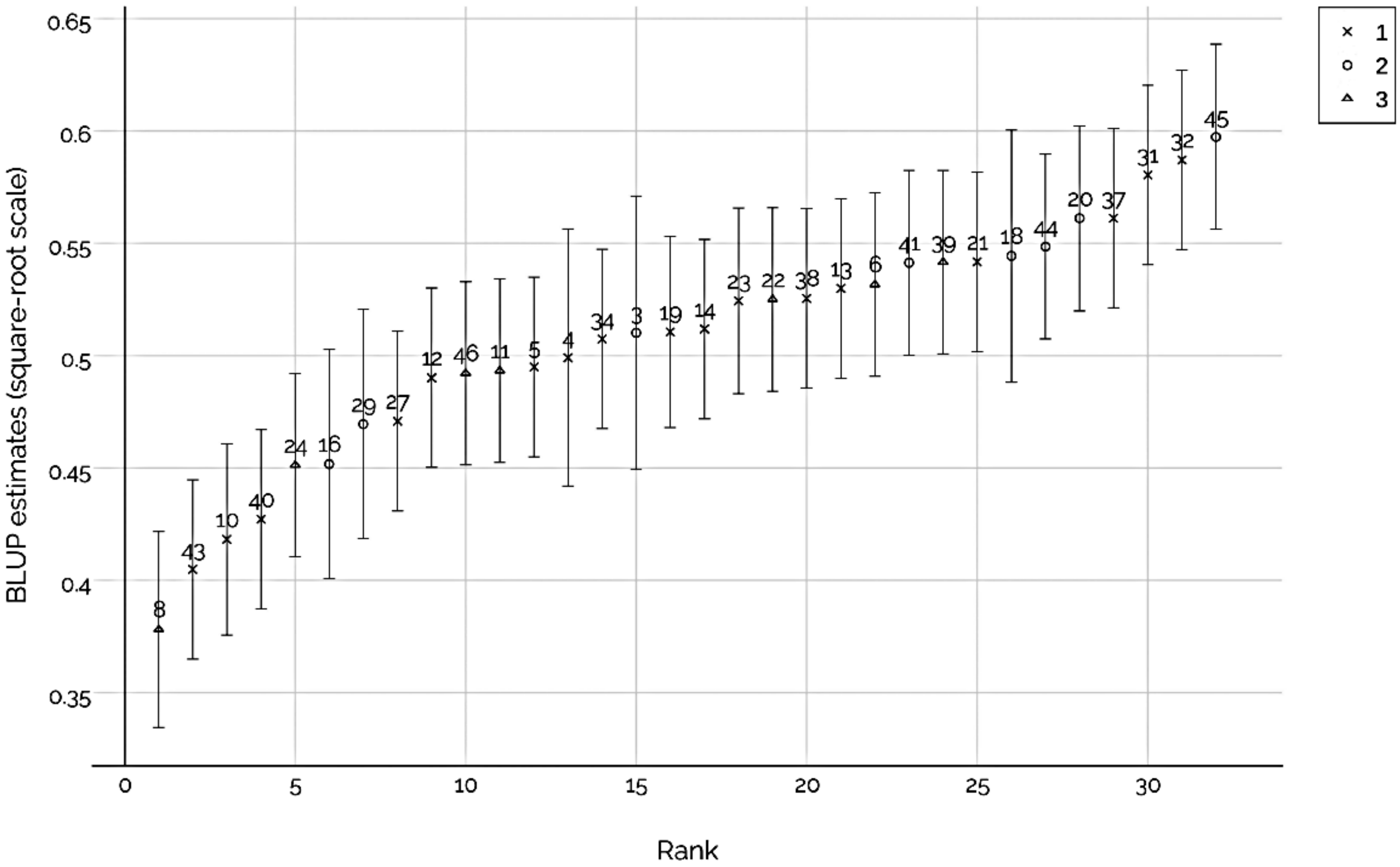
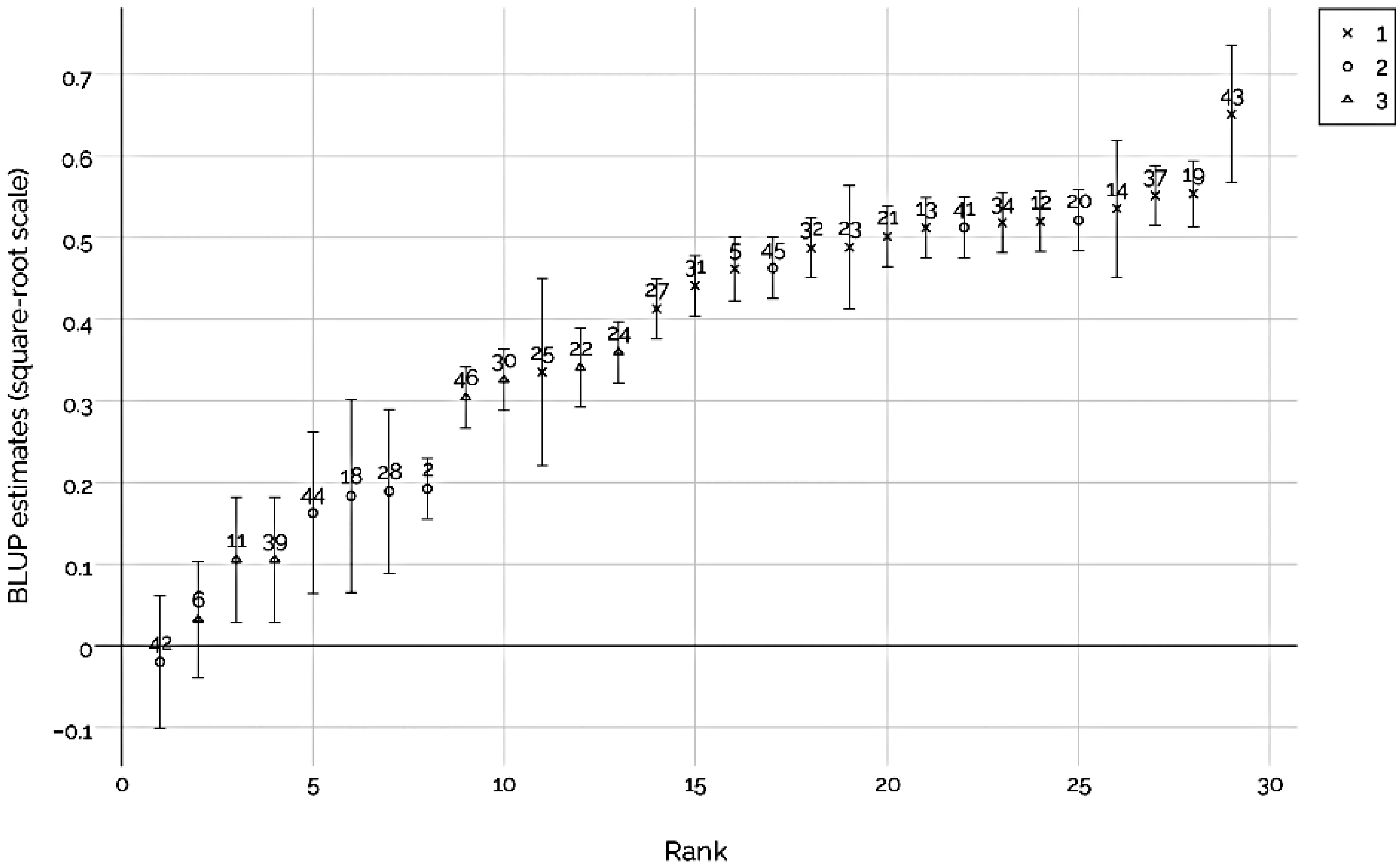


Fig 5.7 Ranking of customers ordering longsnout catfish by best linear unbiased predictor (BLUP) estimates (with ± SE).



5.8. Supplementary materials for Chapter 5

Table S5.1 Fixed effects and random effects estimated by modeling with heterogenous autoregression covariance structure for data of the 3 fish species: sensitivity analysis with ideal Boxcox transformation (indicated by λ) of the outcome variable.

	Largemouth bass (*Micropterus salmoides*) (λ_1=0.47)				**Chinese perch** (*Siniperca chuatsi*) (λ_2=0.55)				**Longsnout catfish** (*Leiocassis longirostris*) (λ_3=0.50)			
Fixed effect	**Estimate**	**95% CI*[a]**			**Estimate**	**95% CI**			**Estimate**	**95% CI**		
Intercept	0.361	0.298 - 0.424			0.474	0.405 - 0.542			0.499	0.406 - 0.591		
market2	0.039	-0.061 - 0.138			0.018	-0.090 - 0.125			-0.222	-0.375 - -0.070		
market3	0.023	-0.076 - 0.122			-0.017	-0.124 - 0.089			-0.273	-0.428 - -0.119		
Random effects	**Estimate**	**SE**	**VPC*[b]**	**ICC*[c]**	**Estimate**	**SE**	**VPC**	**ICC**	**Estimate**	**SE**	**VPC**	**ICC**
$\sigma^2_{customer}$	0.004	0.002			0.005	0.002			0.019	0.007		
$\sigma^2_{market-week}$	0.011	0.003			0.014	0.003			0.010	0.003		
Week (15) σ^2_{k15}	0.023	0.007	0.116	0.162	0.025	0.007	0.107	0.155	0.029	0.011	0.336	0.401
Week (16) σ^2_{k16}	0.004	0.001	0.228	0.514	0.006	0.002	0.190	0.424	0.014	0.006	0.454	0.579
Week (18) σ^2_{k18}	0.007	0.002	0.200	0.389	0.025	0.007	0.108	0.157	0.006	0.002	0.567	0.777
Week (19) σ^2_{k19}	0.011	0.003	0.166	0.280	0.012	0.004	0.156	0.284	0.008	0.003	0.537	0.721
Week (20) σ^2_{k20}	0.012	0.004	0.165	0.276	0.006	0.002	0.195	0.449	0.004	0.002	0.601	0.843
Week (21) σ^2_{k21}	0.007	0.003	0.197	0.380	0.006	0.002	0.191	0.427	0.013	0.005	0.471	0.608
Week (22) σ^2_{k22}	0.010	0.003	0.173	0.299	0.013	0.005	0.148	0.259	0.008	0.003	0.524	0.699
Week (23) σ^2_{k23}	0.006	0.002	0.206	0.412	0.004	0.001	0.213	0.555	0.007	0.003	0.548	0.742
Week (24) σ^2_{k24}	0.007	0.002	0.197	0.380	0.009	0.003	0.170	0.336	0.002	0.001	0.626	0.893
Week (25) σ^2_{k25}	0.016	0.005	0.144	0.222	0.013	0.004	0.150	0.267	0.024	0.009	0.366	0.443
Week (26) σ^2_{k26}	0.023	0.006	0.116	0.161	0.008	0.002	0.176	0.360	0.010	0.004	0.504	0.664

Week (27) σ^2_{k27}	0.068	0.022	0.054	0.062	0.033	0.009	0.091	0.124	0.012	0.005	0.475	0.615
Week (28) σ^2_{k28}	0.008	0.003	0.193	0.363	0.004	0.001	0.214	0.566	0.015	0.007	0.443	0.562
Week (29) σ^2_{k29}	0.013	0.004	0.158	0.257	0.010	0.003	0.164	0.314	0.007	0.003	0.548	0.743
Week (30) σ^2_{k30}	0.039	0.012	0.082	0.102	0.022	0.007	0.115	0.172	0.009	0.004	0.514	0.680
Week (31) σ^2_{k31}	0.004	0.002	0.229	0.517	0.035	0.011	0.088	0.117	0.031	0.013	0.324	0.383
autocorrelation	0.194	0.069			0.288	0.088			0.246	0.094		

Note: *[a] Confidence interval *[b]. VPC, Variance partition coefficient; *[c]. ICC, Intraclass correlation coefficient.

Table S5.2 Fixed effects and random effects estimated by modeling with heterogenous autoregression covariance structure for data of the 3 fish species: sensitivity analysis with square-root transformation the outcome variable and removal of outliers.

	Largemouth bass (*Micropterus salmoides*)		**Chinese perch** (*Siniperca chuatsi*)		**Longsnout catfish** (*Leiocassis longirostris*)	
Fixed effect	**Estimate**	**95% CI*[a]**	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Intercept	0.341	0.279 - 0.403	0.506	0.442 - 0.571	0.499	0.406 - 0.591
market2	0.052	-0.045 - 0.149	0.023	-0.077 - 0.123	-0.222	-0.375 - -0.070
market3	0.025	-0.071 - 0.121	-0.017	-0.115 - 0.082	-0.273	-0.428 - -0.119

Random effects	**Estimate**	**SE**	**VPC*[b]**	**ICC*[c]**	**Estimate**	**SE**	**VPC**	**ICC**	**Estimate**	**SE**	**VPC**	**ICC**
$\sigma^2_{customer}$	0.004	0.002			0.002	0.002			0.019	0.007		
$\sigma^2_{market-week}$	0.011	0.003			0.013	0.003			0.010	0.003		
Week (15) σ^2_{k15}	0.020	0.006	0.108	0.158	0.029	0.009	0.054	0.077	0.029	0.011	0.327	0.400
Week (16) σ^2_{k16}	0.004	0.001	0.202	0.497	0.007	0.003	0.107	0.252	0.014	0.006	0.440	0.579
Week (18) σ^2_{k18}	0.007	0.002	0.174	0.357	0.034	0.010	0.049	0.066	0.006	0.002	0.549	0.776
Week (19) σ^2_{k19}	0.010	0.003	0.150	0.270	0.016	0.005	0.078	0.133	0.008	0.003	0.520	0.721
Week (20) σ^2_{k20}	0.011	0.003	0.146	0.257	0.009	0.003	0.101	0.218	0.004	0.002	0.582	0.843
Week (21) σ^2_{k21}	0.007	0.003	0.171	0.344	0.005	0.002	0.117	0.311	0.013	0.005	0.457	0.608
Week (22) σ^2_{k22}	0.010	0.003	0.152	0.276	0.019	0.006	0.069	0.110	0.008	0.003	0.508	0.698
Week (23) σ^2_{k23}	0.007	0.002	0.175	0.361	0.003	0.001	0.130	0.419	0.007	0.003	0.531	0.740
Week (24) σ^2_{k24}	0.007	0.002	0.170	0.339	0.012	0.004	0.089	0.168	0.002	0.001	0.607	0.893
Week (25) σ^2_{k25}	0.014	0.004	0.129	0.207	0.018	0.006	0.073	0.118	0.024	0.009	0.356	0.443
Week (26) σ^2_{k26}	0.022	0.006	0.103	0.148	0.010	0.003	0.096	0.195	0.010	0.004	0.489	0.663
Week (27) σ^2_{k27}	0.065	0.021	0.047	0.055	0.038	0.010	0.045	0.059	0.012	0.005	0.461	0.614
Week (28) σ^2_{k28}	0.009	0.003	0.161	0.307	0.004	0.002	0.123	0.357	0.015	0.007	0.430	0.561

Week (29) σ^2_{k29}	0.002	0.001	0.220	0.626	0.011	0.004	0.093	0.184	0.007	0.003	0.532	0.742
Week (30) σ^2_{k30}	0.037	0.011	0.073	0.093	0.006	0.002	0.112	0.279	0.009	0.004	0.498	0.679
Week (31) σ^2_{k31}	0.004	0.002	0.196	0.465	0.043	0.013	0.041	0.053	0.031	0.013	0.315	0.383
autocorrelation	0.178	0.067			0.441	0.123			0.246	0.094		

Note: *[a] Confidence interval *[b]. VPC, Variance partition coefficient; *[c]. ICC, Intraclass correlation coefficient.

Chapter 6 Conclusions

6.1. Summary of findings and significance

6.1.1. Background and context

China leads the global production, processing, and consumption of freshwater cultured fish (FAO, 2014). In this country, as in other countries, aquaculture is the fastest-growing food sector, which could be an impediment to the resilience of the national food system, given the uncertainties of resource availability and climate change (Troell et al., 2014). To enhance the resilience of food security, government policies responsive to local social contexts are key to providing incentives to all stakeholders and the efficient use of resources for aquaculture development (FORHEAD, 2014; Troell et al., 2014).

With the urbanization of an aging population in the modernization of China's economy, most current aquaculture farmers or workers in China will be retired from the sector in 15 years (Godfrey, 2013). The central government is preparing for the increase of scale and mechanization of fish farming operations, which will replace the small-scale farmers who currently produce the vast bulk of China's aquaculture output. Given that epidemiological data have been scarce in the country (FAO, 2005; Tan et al., 2006), including a lack of up-to-date census data on the numbers of farmed fish, what should Chinese aquaculture stakeholders consider in order to prepare for the process of transforming knowledge into policy in order to cope with challenges and threats in the complex, changing socio-economic context? Furthermore, in order to solve fish health problems in intensive aquaculture, what approaches should the Chinese take to unlock the full potential of aquaculture?

6.1.2. Fish diseases and control challenges

Fish diseases and health problems plague the sustainability of Chinese freshwater aquaculture industry (Li et al., 2011). In order to assess the biological and socio-economic impacts of disease, specification of species and culture systems is necessary (Fish et al., 2011; Peeler and Taylor, 2011). More than 60% of Chinese warm-water production is carp species targeted for domestic markets and, more recently, the farming of high-value carnivorous freshwater finfish species has been widely promoted and accepted across the whole freshwater finfish industry in China (Wang et al., 2014). Due to the diversity of species and variations of socio-economic and environmental characteristics of aquaculture systems in China, little aquatic epidemiology research from other areas in the world can be used to explore the distribution of and risk factors for fish diseases in warm-water aquaculture in China.

Compared with the impacts of uncontrollable external factors, such as resource scarcity, natural disaster, and instability of prices, the impacts of aquatic infectious diseases can be mitigated if epidemiological understanding is sufficient to put prevention and control measures in place (Bondad-reantaso, 2005). Due to the difficulties of ascertaining the underlying causes of aquatic disease outbreaks, aquatic epidemiology, as a holistic health management approach (Subasinghe, 2005), can delineate the temporal, spatial, and demographic dynamics of the endemic diseases of farmed fish (Perez, 2015).

However, domestic aquaculture academia in China has been more focused on traditional “blue-sky” research into biological understanding of pathogens at microscopic levels, rather than the bigger context of fish health problems at the population level and how anthropogenic factors influence the impacts of fish health problems (Li et al., 2016; Peng, 2013; Xie et al., 2015). Furthermore, due to the absence of aquatic disease epidemiology in the educational curriculum and lack of epidemiologists in this field, standardized guidelines and protocols are not available for the collection and analysis of field data related to fish mortality and health problems. What, then, should be the starting points for the paradigm shift to accommodate new, evidence-based ways of thinking about disease control concepts and approaches?

6.1.3. Summary of research findings

Based on our experience with (unpublished) trial surveys of carp farmers in 2012 and 2013 in Hubei Province, I focused on two questions of interest in our interviews of fish farmers culturing yellow catfish in 2 Chinese provinces: (1) Under what circumstances can fish farmers make a profit? and (2) How do those farmers implement fish health management practices? I found that carp aquaculture in both provinces was financially viable, with mean returns-costs ratios of 1.31 and 1.17 for Guangdong and Zhejiang, respectively (Chapter 2). Guangdong farmers spent more money on fixed and variable costs (i.e. land and electricity) than farmers in Zhejiang, even though they achieved better returns-costs ratios. High costs of feed and land rent negatively affected net returns in both provinces, and the market price of fish was also an influential factor in net returns (Chapter 2). I quantified behaviors and perceptions of fish farmers, and found biosecurity was a new concept for many respondents. Farmers' practices were, overall, not compliant with established biosecurity principles, especially in regards to prevention of pathogen introduction and spread (Chapter 3). However, farmers were open to improvement to current health management practices, with the goal of effectively preventing losses caused by fish diseases (Chapter 3).

Using farm production records, I found predisposing factors that could affect the dynamics of daily mortality counts of farmed grass carp (Chapter 4). There was uncertainty of the impacts of those predisposing factors among ponds on the same farms (Chapter 4). Not all current treatments can reduce grass carp mortalities, and effective management practices need to be in place minimize the negative effects of those factors (Chapter 4).

As to the marketing of live fish, I found that the variations in mortality claims of transported live fish (Chapter 5) were species dependent. Customers who ordered longsnout catfish had more variations in claims than those ordering largemouth bass and Chinese perch (Chapter 5). I also found the possibility of variations in mortality claims being attributed to delivery factors, such as health status of transported fish, water quality management, and packaging conditions. These findings indicate a need for better customer communications and improved technical parameters for live fish transportation (Chapter 5).

6.1.4. Knowledge gap that the thesis has filled

In order to act as a bridge in the collection and dissemination of knowledge between farmers and decision-makers, I focused on the different stakeholders along the food chain of freshwater fish, including fish farmers, fish logistics companies, and aquatic feed companies. The research chapters of this thesis provide insight into aquatic epidemiological methods best suited to tackle the health management problems in warm-water aquaculture in China for different stakeholders, from farmers to live fish transporters (Fish et al., 2011; Krause et al., 2015).

Aquaculture production has unique challenges for economic analyses, and few publications have demonstrated detailed applications of economic models in aquaculture, especially using "on-the-ground" farm survey data (Engle, 2010). One of the challenges is that key costs might be inadvertently ignored during the basic budget analysis using farm-level data. To provide Chinese fish farmers with a useful budget analysis method, I developed a spreadsheet for budget analysis in Excel, which could be developed as a cellphone app, web-based spreadsheet, or another format, depending on the needs of the end-user. The Excel-based budget analysis tool is also useful to increase the transparency of budget information-sharing between feed company staff and fish farmers. Our tool incorporates mortality so it can be used to assess whether or not new farm management practices that improve biosecurity and potentially reduce mortality are cost effective over time.

The knowledge-attitudes-practices (KAP) survey of farmers' behaviors and perceptions of fish farm biosecurity in this thesis is the first study of biosecurity in small-scale, freshwater pond aquaculture in China. This study described farmers' acceptance of aquatic biosecurity measures by individuals producing warm-water fish in China, and also highlighted for Chinese policy-makers and researchers the gaps in biosecurity knowledge, attitudes, and practices that may need to be closed in order to transform farms into intensive fish rearing facilities. The methodology detailed in the study could provide points of reference for future research using fish farmers' surveys in China, in terms of exploring the correlations between their perceptions of disease and fish health management practices (Ajzen, 2011; Cliff and Campbell, 2012; Frössling and Nöremark, 2016).

Mortality information on freshwater fish, especially pond aquaculture, has been regarded as having limited value (Peeler and Taylor, 2011). This thesis has 2 chapters dealing with fish mortalities recorded at 2 different points along the food chain: farm production and marketing, where fish were not from consecutive or the same epidemiological units.

I chose to study the transaction data from a fish logistics company because the urban markets and urban-rural supply chains are, by far, the most important economic drivers affecting farmers (Belton and Bush, 2014). I used a cross-classified model to explore sources of mortality variation in transported live fish, i.e., species, customers, deliveries, and time. The method is not only useful for fish logistic companies to mitigate risks of fish mortality after collection from the farmers, but is also useful to indicate whether fish health problems might originate at the farm. Our findings highlight the need to improve live transport of fish in China. Although some customers appeared to complain more often than others, there was a high level of mortality amongst the fish evaluated that appeared to be related to the shipments themselves.

Based on theories of fish stress and welfare, I grouped atmospheric temperature, treatments, and movement of fish as factors that can cause stress. To our knowledge, this is the first application of a time-series regression method to explore the lagged effect of these potential predisposing factors on grass carp mortalities. The study is meaningful, as it identified risk factors of fish health management that might contribute to fish mortality at the farm level. This chapter also indicates the most important health management information that should be included in the daily farm records of fish farmers: daily mortality, movement of fish, chemical treatment details (name and usage of chemical applied), and water quality parameters.

6.2. Challenges and introspective considerations

6.2.1. Lack of fish disease diagnostic data

Fish mortality data used in both statistical modeling chapters did not have laboratory diagnostic test results to determine the causative biological agents associated with these events. This made it difficult to differentiate causes of mortality, which is necessary to identify disease risk factors. Further information, such as fish physiology, transportation conditions, and pond water quality parameters, were not detailed enough to allow us to reach a concrete understanding of their roles in reported mortalities of transported fish or recorded mortalities of farmed fish. Because these data were not available, this study was limited in our ability to assess the information provided to us by farmers. As farms increase their production it will become more important to collect this type of information to help prevent and control infectious and non-infectious causes of fish mortality. Proper disease diagnosis and analysis of diagnostic data is a prerequisite for good understanding of disease epidemiology and effective health management (Gardner et al., 2000; Gardner, 2002; Tan et al., 2006). However, fish diagnostic data rarely accompany fish mortality data at the farm level for warm-water finfish health management in China. Farmers' decisions to use pharmaceuticals are influenced by chemical dealers' promotions and most farmers , based on our surveys, lacked the knowledge to apply chemicals properly (Rico et al., 2012; Zhang, 2014). Even though diagnostic capacities and research potential for fish diseases are being established at the national and provincial levels in China, there are deficiencies in the farm-level data collected by farmers. It would appear from our surveys and interviews that many farmers don't have access to a laboratory and, therefore, base their diagnoses on field observations of clinical signs only (Tan et al., 2006). Another government-funded initiative of web-based diagnostic software, based on expert opinions and established in the early 2000s (Li et al., 2002), might have played a role in knowledge transfer, but the system relied on anecdotal reporting of symptoms without the support of laboratory diagnostic services, which occurred at the expense of the development of laboratory infrastructure for fish disease diagnostics.

Standardization, validation, and inter-calibration of rapid and accurate diagnoses of emerging aquatic diseases will be essential to future, accurate risk management and biosecurity-based management of warm-water finfish in China (Bondad-Reantaso et al., 2005).

6.2.2. Incomplete understanding of fish farmers' perspectives

Our descriptive study on aquatic biosecurity might not fully explain all factors influencing farmers' adoption of biosecurity measures and other aquatic health management practices. Farmers' decision-making could also be explained through social-psychological approaches, and there could be diverse attitudes and beliefs held by producers (Mitchell, 2006; Davidson et al., 2009; Pereira, 2011; Garforth et al., 2013; Toma et al., 2013). For example, most farmers regard farm records as very important, but in reality very few of them kept records of feeding, treatments, or health problems. I also could not match biosecurity data and return-cost analysis results due to a limited number of matching observations from the same farms.

According to anecdotal notes from fish farmers consulted during the meeting organized by the fish transportation company, most farmers would commit to the protocols detailed in the

Good Aquaculture Practices (GAPs) if profitability could be increased by these practices and if it ensured stable and high market prices. This is very similar to the findings from previous studies that deduced that market is important for the development of personal motivation to follow production guidelines of responsible farming of food animals (Gardezi, 2014). The reality is that fish farmers' lack of knowledge of diseases in their production systems might also be a barrier to their understanding of biosecurity or disease prevention plans (Palić et al., 2015).

6.2.3. Generalizability of epidemiological approaches to warm-water finfish species

One might ask whether or not aquatic epidemiology will be a panacea for improved health management of all warm-water finfish species in China? The answer can be yes and no. We may answer "yes" if we consider aquatic epidemiology as a series of holistic and trans-disciplinary approaches to deal with fish health problems and diseases, with the concept that "an ounce of prevention is worth a pound of cure."

However, there are no uniform solutions towards the health management problems for all farmed warm-water finfish. The following components of epidemiological methods might need to be considered, due to the complexities of farmed fish species and their rearing systems (De Blas, 2005; Oidtmann et al., 2013).

- **Study population.** Definition and selection of the study population is critical for epidemiological studies. The study population and selection methods of the prioritized diseases of are related to the structure of fish populations farmed in the same premise, production costs and returns of fish farming, temporal and spatial patterns of fish mortalities, and their dependence on commercial feed.

- **Quality of diagnostic tests.** Selection of diagnostic tests and their performance characteristics need to account for other factors during the process in which the test results are produced, such as the sampling procedures, and applicability of the method in detection of diseases in the field (Adams and Thompson, 2008; Morgan et al., 2015). Molecular diagnostic tests, such as PCR techniques and sequence analysis, as well as serological methods, have been applied in aquatic epidemiological studies; however, rigorous evaluation of their accuracy (sensitivity and specificity) is needed to facilitate sound interpretation of results (Gardner et al., 2014).
- **Characterization of pathogens**. Source of pathogen, host spectrum, drift and shift of pathogen genotypes, incubation period, transmission mechanisms, and presence or absence of the pathogen during different life stages of the fish are characteristics to consider when determining the most appropriate methods of outbreak investigation, surveillance, and epidemiological studies of aquatic diseases for finfish (FAO, 2004).
- **Sampling methodology.** Combined with different objectives of screening and confirmation of fish diseases, sample size, geographical location, sampling methods, and post-sampling procedures are detailed in the guidelines of the World Organisation for Animal Health (OIE, 2015).

6.3. Future work

6.3.1. Consultations with different stakeholders

With new actors involved in knowledge generation and dissemination, knowledge has become practice-related and exchangeable between researchers and their subjects. Current research projects do not stand in isolation as "objective" and "external" investigations (Bosselaar, 2015; Wyatt, 2015), but rather are engaged in conversations with prior research, as they reorder and generate new institutional and social formats for knowledge transfer and application. Not only in China, but also globally, there has been a gap in the transfer of knowledge between stakeholders: companies, policy makers, fish farmers, and consumers (Belton and Bush, 2014). The disconnect between science and policy in aquaculture and the contextual approach of aquaculture research have been neglected at different scales of social impacts (individual, community, national, regional, and international) (Krause et al., 2015). There is a need to improve the process of knowledge dissemination to related stakeholders and the translation of new knowledge into policy. The results from this thesis will be shared among different stakeholders related to warm-water fish aquaculture in China in order to initiate consultations on (1) prioritization of fish health problems and diseases of warm-water finfish; (2) the shared responsibilities reducing social-economic impacts from losses of diseased fish; and (3) pragmatic solutions towards fish health management (Karreman et al., 2015).

6.3.2. Economic analysis of diseases losses

Early-stage quantitative analyses of losses due to animal health problems can be traced back to the late 1990s for terrestrial animal diseases (Bennett et al., 1999) and aquatic animal diseases (Menzies et al., 2002). In terms of assisting effective policy responses and interpreting multiple factors and losses simultaneously, the bottom-up value chain method has proven useful to integrate the epidemiology of animal diseases and economic behaviours (Rich and Perry, 2011; Rich et al., 2013). However, no published studies have reported the economic evaluation of disease control measures for warm-water fish diseases in China. To provide evidence of the usefulness of aquatic epidemiological approaches, i.e. biosecurity measures and other basic sanitation practices, there is a need to demonstrate to stakeholders the monetary value of the adoption of evidence-based, proactive measures to control fish disease and health problems.

Many mortality events appear to go undiagnosed in small-scale aquaculture in China. Our understanding of the knowledge, attitudes, and practices of the farmers is limited, and the economics of their farm situations likely limit the biosecurity practices on farms. Transportation of fish from farms to markets can be problematic and may present another set of health issues during live transportation. As China's aquaculture industry moves from extensive to intensive there will have to be increased and improved fish health practices to prevent and control infectious disease outbreaks. This will require better data collection, as many risk factors for disease prevention in warm-water finfish culture in China are unknown. The work described in this thesis may provide a point of departure for the development of holistic, epidemiological approaches to fish health management in China. Given current work of field surveys in the 2 Chinese provinces, two types of studies are likely to be most useful to evaluate the impacts of diseases and control measures on the economics of warm-water finfish farming. The first is a study to assess general health management strategies of farmed and transported fish populations. The second is a study of indirect costs of production and marketing; for example, the costs to farmers of vaccinating (or not) against grass carp hemorrhagic virus.

6.4. References

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